THE UNIVERSITY
of EDINBURGH

The role of systemic inflammation in cerebral small vessel disease.

Stewart John Wiseman

Doctor of Philosophy

The University of Edinburgh

2016

ABSTRACT

Cerebral small vessel disease (SVD) is a distinct microvascular disorder that can lead to lacunar stroke, an important stroke subtype that accounts for a quarter of all ischaemic strokes. SVD is associated with imaging biomarkers such as white matter hyperintensities (WMH). The cause of SVD is largely unknown, although inflammation and blood-brain barrier failure via endothelial dysfunction have been implicated. Elevated plasma biomarkers of inflammation are associated with coronary heart disease and large vessel stroke but the role of inflammation in SVD is less well understood. Our hypothesis is that inflammation plays a role in SVD and we sought to examine this by reviewing the literature for evidence of this, and by conducting a brain imaging study of patients with a known inflammatory disease and reviewing the images for evidence of inflammation and SVD, and comparing findings with controls groups.

Section A: This thesis begins with a systematic review and meta-analysis of 13 plasma biomarkers of four physiological processes (coagulation, fibrinolysis, endothelial dysfunction and inflammation) in lacunar stroke versus non-lacunar stroke (to control for having any stroke) and non-stroke (to compare to the general population). We sought to know if there were differences in these biomarkers between lacunar stroke and other stroke subtypes and non-stroke controls as a way of generating hypotheses for the disease mechanisms that might lead to lacunar stroke. Findings revealed differences in several biomarkers between lacunar stroke and healthy controls but only fibrinogen, D-dimer, von Willebrand factor and interleukin-6 were different (all significantly lower in lacunar stroke) between lacunar stroke and other stroke subtypes. There was heterogeneity between studies, including variations in the definition of lacunar stroke and most studies

measured the biomarkers in the acute phase post stroke, which is potentially confounding. To further examine plasma biomarkers of inflammation and endothelial dysfunction in SVD, we used data from a prior study of mild stroke conducted at the Brain Research Imaging Centre, University of Edinburgh, UK. Lacunar stroke patients were compared to cortical stroke patients. The lacunar group had lower levels of tissue plasminogen activator independent of age, sex and vascular risk factors but we found no difference in the other plasma biomarkers.

Section B: Non-resolving systemic inflammation is a feature of inflammatory autoimmune rheumatic diseases such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE). These patients are at increased risk of stroke but much knowledge relates to stroke in general; less is known about associations with stroke subtypes including SVD, or when in life stroke risk is greatest. Consequently, we sought to better understand the influence of inflammatory rheumatic diseases on stroke and SVD. The review and meta-analysis of cerebrovascular disease in rheumatic diseases showed an excess risk of stroke in RA, SLE, ankylosing spondylitis, gout and psoriasis over the general population. Meta-analyses of stroke subtypes (ischaemic and haemorrhagic) in RA and SLE showed an excess risk of stroke over the general population. Stroke risk across rheumatic diseases was highest in those aged <50 years and reduced with ageing. We then requested data from NHS Lothian covering 15 years so that we could assess stroke, including stroke subtypes, among patients diagnosed with various arthropathies. We linked 6,613 rheumatology patients' records with stroke admission records, grouped the various rheumatic diseases into the two main types of arthritis, inflammatory and non-inflammatory, and also compared the strokes in these rheumatology patients to general population data. There was no difference in stroke prevalence between inflammatory and

degenerative (non-inflammatory) arthropathies, although the strokes occurred up to two decades earlier than in the general population.

Section C: Lastly, we conducted MRI neuroimaging in patients with SLE and reviewed and meta-analysed diffusion tensor imaging (DTI) (an imaging technique used to assess sub-visible white matter microstructure damage) in SLE to place our findings into context. The research question here was to ascertain if patients with a known inflammatory disease had brain imaging evidence of SVD, and to compare findings to controls. We compared imaging markers of SVD and DTI between SLE patients and age-matched healthy controls and sought associations between the imaging biomarkers and plasma biomarkers of inflammation and endothelial dysfunction, measures of fatigue and cognition, and scores of rheumatic disease activity. Fifty-one patients were recruited. There was higher mean diffusivity in all white matter tracts versus controls indicating a diffuse increase in brain water mobility in SLE. Meta-analysis confirmed higher mean diffusivity in SLE patients versus controls. Fatigue in SLE was significantly higher than a normal reference range and was associated with depression, anxiety, higher body mass index, lower mean diffusivity and some blood markers of inflammation and endothelial dysfunction. The most fatigued were youngest which explained the association with lower mean diffusivity. Damage to the brain's white matter microstructure may be accelerated in SLE as the age-related declines in the general population are normally seen much later in life. The aging pattern is consistent with inflammation-related microvascular-mediated brain damage where the inflammation is systemic in origin.

Summary: This thesis has demonstrated an increase in SVD burden in the inflammatory rheumatic disease SLE and increased stroke risk at younger ages in other inflammatory rheumatic diseases. Thus, systemic inflammation as seen in inflammatory rheumatic

diseases could have effects on the brain directly, including influencing stroke risk which is clinically noteworthy and would benefit from further testing in appropriately designed studies such as an inception cohort that follows inflammatory rheumatic patients from diagnosis, with regular brain imaging to track brain changes and correlates with inflammatory profiles and impact on cognition.

LAY SUMMARY

Cerebral small vessel disease (SVD) is a disease of the blood vessels in the brain that can lead to stroke. The cause of SVD is largely unknown, although inflammation has been implicated. Elevated blood markers of inflammation play a role in heart disease but the role of inflammation in SVD is less well understood.

Section A: This thesis begins with a review of the scientific literature of molecules found in blood tests between lacunar stroke (an important type of stroke) and other types of stroke, and with healthy people that have not had a stroke. It revealed differences in several of these molecules which helps scientists understand why and how different types of strokes might happen. However, there were differences in the quality of the studies and the methods they used which limits how much we can infer from them. Most of the studies drew blood too soon after stroke when many biological processes are likely to be highly active because of the stroke and this limits what scientists can infer. To further examine inflammation in SVD we used data from a prior study of stroke conducted at our Centre. Lacunar stroke patients were compared to another type of stroke. The lacunar group had lower levels of one type of blood biomarker (tissue plasminogen activator) independent of age, sex and risk factors for stroke such as high blood pressure but we found no difference in the other biomarkers.

Section B: Patients with inflammatory arthritis (e.g. rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE)) are at increased risk of having a stroke. However, much knowledge relates to stroke in general and less is known about lacunar stroke or when during the course of a person's life stroke is most likely to happen. Our review of the scientific literature of stroke and SVD in arthritis showed that people diagnosed with RA and SLE as well as other types of arthritis such as ankylosing spondylitis, gout and psoriasis were more likely to suffer a stroke. Stroke risk across different types of arthritis was highest in those aged <50 years. We then requested data from NHS Lothian covering 15 years so that we could look at stroke among a local group of patients diagnosed with various types of arthritis. We linked 6,613 rheumatology patients' records with stroke admission records, grouped the various types of arthritis into 'inflammatory' and 'non-inflammatory' and also compared the strokes to the general population (i.e., people with no arthritis). There was no difference in the number of strokes occurring between inflammatory (e.g., RA) and degenerative arthritis (e.g., osteoarthritis) although the strokes in the arthritis patients occurred up to two decades earlier than in the general population meaning patients with arthritis are at risk of stroke earlier in life which was a similar conclusion with our other study.

Section C: Lastly, we undertook a brain imaging study (using MRI) with patients diagnosed with SLE. We reviewed the scientific literature for one type of advanced neuroimaging technique used to see the tiny white matter fibres in the brain. We looked at a range of measurements taken from the brain scans between the patients with SLE and other groups of people. We also looked at fatigue and cognitive abilities in these patients in relation to the brain scans. Fifty-one patients were recruited. The 'wiring' (the brain's

white matter) of the brain in the SLE patients was more disorganised compared to the people who did not have SLE. Our review of the literature confirmed this finding. Fatigue in SLE was significantly higher than would have been expected if you test a group of people at random and was related with depression, anxiety, higher body mass index and inflammation. We concluded that damage to the brain's white matter may be accelerated in SLE. The age related changes were consistent with the picture that emerged from our earlier work.

DECLARATION

I declare that this thesis has been composed by me. Co-authored publications are identified below – my contribution to these was substantial, including: conception, design, data acquisition, analysis, drafting, critical revision for important intellectual content and accountability for accuracy and integrity of content, as per the International Committee of Medical Journal Editor's definition of authorship.

The work presented in Chapter 2 was published in 2014 in *Cerebrovascular Diseases* as "Blood markers of coagulation, fibrinolysis, endothelial dysfunction and inflammation in lacunar stroke versus non-lacunar stroke and non-stroke: systematic review and meta-analysis" by Wiseman SJ, et al. I contributed to the design of the work, conducted the review, analysed the data, meta-analysed the results, and drafted and edited the paper. Others contributed to the design of the study, the search strategy and the editing of the final manuscript. We received approval on 14th March 2016 from S. Karger AG, the publishers, for the paper to be reproduced in this thesis (a full citation is given in the next section).

The work presented in Chapter 3 was published in 2015 in *Cerebrovascular Diseases* as "Plasma biomarkers of inflammation, endothelial function and haemostasis in cerebral small vessel disease" by Wiseman SJ, et al. I undertook some of the image analysis, conducted all the statistical analysis and drafted and edited the paper. The data came from a prior study at our Centre, which was designed and conducted by others. Others also helped edit the final draft. We received approval on 14th March 2016 from S. Karger AG, the publishers, for the paper to be reproduced in this thesis (a full citation is given in the next section).

The work presented in Chapter 4 was published in 2016 in Stroke as "Cerebrovascular disease in rheumatic diseases: A systematic review and meta-analysis" by Wiseman SJ et al. I contributed significantly to the conception and design of the study. I conduced the review, extracted the data

and performed all analyses. I drafted and edited the manuscript. Others helped with drafting and final editing of the paper. The publishers, Wolters Kluwer, confirmed to me on 14th March 2016 that they allow free reproduction to authors of published papers for inclusion in a PhD thesis (a full citation is given in the next section).

The work presented in Chapters 6, 7 and 8 has been accepted for publication as "Fatigue and cognition in systemic lupus erythematosus: associations with white matter microstructural damage. A diffusion tensor MRI study and literature review" by Wiseman SJ et al. and "Small vessel disease burden is increased in systemic lupus erythematosus" by Wiseman SJ et al. Each chapter contains substantial input from me, from study conception and design to grant application to ethics approval to study execution to drafting of the final papers. Others helped with study conception and design, the grant application, patient recruitment and drafting of the papers.

This thesis has not been submitted for any other degree or professional qualification.

Signed: _____ Stewart John Wiseman

Dated: _____

PUBLICATIONS RELATED TO THIS THESIS

Wiseman SJ, Ralston SH, Wardlaw JM. **Cerebrovascular disease in rheumatic diseases: systematic review and meta-analysis.** *Stroke* 2016;47:943–50.

Wiseman SJ, Doubal FN, Chappell FM, Valdes-Hernandez MC, Wang X, Rumley A, Lowe GDO, Dennis MS, Wardlaw JM. **Plasma biomarkers of inflammation, endothelial function and hemostasis in cerebral small vessel disease.** *Cerebrovasc Dis* 2015;40:157–64.

Valdes-Hernandez MC, Maconick LC, Maniega SM, Wang X, Wiseman SJ, Armitage PA, Doubal FN, Makin S, Suddlow CLM, Dennis MS, Deary IJ, Bastin M, Wardlaw JM. **A comparison of location of acute symptomatic vs. 'silent' small vessel lesions.** *Intl J Stroke* 2015;10:1044–50.

Wiseman SJ, Marlborough F, Doubal F, Webb DJ, Wardlaw JM. **Blood markers of coagulation, fibrinolysis, endothelial dysfunction and inflammation in lacunar stroke versus non-lacunar stroke and non-stroke: systematic review and meta-analysis.** *Cerebrovasc Dis* 2014;37:64–75.

Aribisala BS, Wiseman SJ, Morris Z, Valdes-Hernandez MC, Royle NA, Maniega SM, Gow AJ, Corley J, Bastin ME, Starr J, Deary IJ, Wardlaw JM. **Circulating inflammatory markers are associated with magnetic resonance-visible perivascular spaces but not directly with white matter hyperintensities.** *Stroke* 2014;45:605–7.

Submitted to peer-reviewed journals:

Wiseman SJ, Bastin ME, Hamilton IF, Hunt D, Ritchie SJ, Amft N, Thomson S, Belch JFF, Ralston SH, Wardlaw JM. **Fatigue and cognition in systemic lupus erythematosus: associations with white matter microstructural damage. A diffusion tensor MRI study and literature review.** [Accepted for publication in *Lupus* 16/8/16]

Wiseman SJ, Bastin ME, Jardin CL, Barclay G, Hamilton IF, Sandeman E, Hunt D, Amft N, Thomson S, Belch JFF, Ralston SH, Wardlaw JM. **Cerebral small vessel disease burden is increased in systemic lupus erythematosus.** [Accepted for publication in *Stroke* 24/8/16]

ACKNOWLEDGEMENTS

I would like to offer my sincere and grateful thanks to Professor Joanna Wardlaw CBE without whom the whole process of doing a PhD would have been very much harder. Her support, advice, expert opinion and feedback on my written work were simply invaluable. I would also like to acknowledge her exceptionally diligent assistant Kirsten Shuler who helped me in so many ways. My second supervisor, Professor Stuart Ralston, gave his knowledge on rheumatic diseases, provided speedy feedback on my papers and helped and encouraged me throughout. Warm thanks to Dr Nicole Amft and Susan Thomson from rheumatology; both were wonderful ambassadors for the systemic lupus erythematosus (SLE) brain imaging study which made subject recruitment so much easier. I thoroughly enjoyed visiting them and discussing the project. I am indebted to Dr Mark Bastin for the white matter tractography data.

I thank my MRI research radiography colleagues from the Brain Research Imaging Centre (BRIC), Elaine Sandeman, Iona Hamilton, Gayle Barclay, Charlotte Jardin and Donna McIntyre who not only scanned and helped with cognitive testing and image analysis on the SLE study, but also for listening to me moan about thesis writing. The administrative staff (Dr Duncan Martin and Moira Henderson) and my other colleagues at BRIC are also acknowledged, particularly our statistician Dr Francesca Chappell who encouraged me to use R and avoid p values, and also Dr Dominic Job, Dr Maria Valdes Hernandez, Dr Stephen Makin, Dr David Hunt and, from the Centre for Cognitive Ageing and Cognitive Epidemiology, Dr Stuart Ritchie.

I thank the patients with SLE who gave their time and shared their stories. Clinical research is not possible without such generosity.

Lastly, the most important people: mum, dad, friends and fellow yogis.

LIST OF MAIN ABBREVIATIONS

ACER – Addenbrooke's cognitive examination – revised

AS – ankylosing spondylitis

AF – atrial fibrillation

BBB – blood-brain barrier

BMI – body mass index

BILAG – British Isles lupus assessment group 2004

BRIC – brain research imaging centre

BTV – brain tissue volume

CI – confidence interval

CRP – C-reactive protein

CSF – cerebrospinal fluid

DT-MRI – diffusion tensor magnetic resonance imaging

FLAIR – fluid attenuated inversion recovery

FA – fractional anisotropy

FSS – fatigue severity scale

GRE – gradient-recalled echo

HADS – hospital anxiety and depression scale

ICAM – intercellular adhesion molecule-1

IL-6 – interleukin-6

ICD-10 – International Classification of Diseases

ICH – intracerebral haemorrhage

ICV – intracranial volume

MD – mean diffusivity

MSS – Mild Stroke Study

MSS 2 – Mild Stroke Study 2

MMSE – mini mental state examination

MoCA – Montreal cognitive assessment

MR/MRI – magnetic resonance imaging

NART – national adult reading test

NPSLE – neuropsychiatric systemic lupus erythematosus

PAI – plasminogen activator inhibitor

PsA – psoriatic arthritis

PVS – perivascular spaces

ROI – region of interest

REC – research ethics committee

RA – rheumatoid arthritis

SLEx – Scottish Lupus Exchange Registry

SLE – systemic lupus erythematosus

SLEDAI/SLEDAI-2K– systemic lupus erythematosus disease activity index 2000

SLICC – systemic lupus international collaborating clinics

SMD – standardised mean difference

t-PA – tissue plasminogen activator

TIA – transient ischaemic attack

TOAST – Trial of Org 10172 in Acute Stroke Treatment

TNF-α – tumour necrosis factor alpha

WMH – white matter hyperintensities

VCAM – vascular cellular adhesion molecule-1

vWF – von Willebrand factor

TABLE OF TABLES

TABLE OF FIGURES

TABLE OF CONTENTS

Chapter 1: General introduction and aims of thesis

Stroke – a global health problem

Stroke is a key global health issue of growing importance in ageing populations and with increasing socioeconomic affluence among the developing economies[1,2]. In high-income countries, stroke has overtaken all cancers combined to become the second commonest cause of death behind heart disease, while in low-income countries stroke is in the top four killers[3,4]. The risk of stroke deaths in adults aged 20–64 years is lower than that in older adults, but there was a 37% increase in the number of total stroke deaths among younger adults in developing countries between 1990 and 2013[5]. In 2010, the absolute number of people with first stroke (16.9 million), stroke survivors (33 million) and stroke-related deaths (5.9 million) were high and had significantly increased since 1990 (68%, 84% and 26% increase, respectively)[2,4]. In the US, the direct healthcare costs and lost productivity costs of stroke are estimated at $34 billion each year[6]. Stroke is the leading cause of worldwide adult disability and so primary prevention is a public health priority in most countries, particularly as many strokes are likely to be avoided via lifestyle choices that reduce risk factors[7,8].

Stroke and stroke subtypes

A stroke is a sudden brain injury caused by an interruption to cerebral blood flow with symptoms lasting more than 24 hours[9,10]. The resultant focal neurological deficit can present symptomatically as loss of speech, loss of limb function, etc, depending on the region and amount of brain affected. A transient ischaemic attack, or 'mini stroke', exhibits the same signs and symptoms as stroke, but these resolve within 24 hours.

There are two main stroke subtypes: ischaemic stroke and haemorrhagic stroke. Ischaemic strokes are commonest, accounting for about 80% of new strokes. Such strokes result from ischaemia, i.e., a restriction of cerebral blood flow caused by stenosis (narrowing) due to plaque build-up within vessels supplying blood to the brain, or occlusion (partial or full blockage) due to emboli from the heart, aorta or common carotid arteries, or arising locally within the cerebral circulation (thrombi). Emboli and thrombi are also known as clots. The loss of blood supply to brain tissue results in an infarct. The extent of brain damage will depend on the location of the blockage and extent of the ischaemia. Ischaemia is seldom complete and collateral perfusion can keep cells viable[11,12]. Reperfusion can occur spontaneously as clots naturally break up. This process can be accelerated by thrombolysis therapy, now used routinely to treat acute ischaemic stroke, or by mechanical clot retrieval. Some infarcts can be silent[13]. Ischaemic strokes can be further subtyped: half are caused by large artery atheroma, 20% by cardioembolism, 25% are classified as lacunar (see below) and the remainder result from rare causes[14]. Haemorrhagic stroke, also known as intracerebral haemorrhage (ICH), results from a tear or dissection in the wall of a blood vessel and accounts for 10-15% of strokes. The remaining strokes are subarachnoid haemorrhage in type (about 3-5% of all strokes).

Lacunar stroke, an important clinical manifestation of cerebral small vessel disease (SVD)

Lacunar stroke is a distinct stroke subtype, presenting with distinct clinical features, and is classified according to how the symptoms manifest as per the classical clinical lacunar syndromes, being: pure motor weakness (in two areas, e.g., face and arm); sensory loss; combined motor-sensory loss; ataxic hemiparesis or clumsy hand

dysarthria syndrome[15]. It can be caused by ischaemia or haemorrhage, and when ischaemic the size of the infarcts are usually < 20 mm in axial diameter and located in the subcortical parts of the brain supplied by the perforating cerebral arterioles[16–19]. About 10% of lacunar ischaemic strokes are cardioembolic or atherothromboembolic – the rest are presumed due to cerebral small vessel disease (SVD).

Risk factors for lacunar stroke include age, hypertension, diabetes, hyperlipidemia and smoking, although non-lacunar strokes are equally associated with many of these risks[20].

Clinical lacunar ischaemic stroke is associated with imaging features of SVD (see Table 1.1 and Figure 1.1 overleaf) being any of: recent small subcortical infarcts (RSSI), white mater hyperintensities (WMH), lacunes, enlarged perivascular spaces (PVS) and cerebral microbleeds, more than are other stroke subtypes. Imaging features of SVD were recently formalised in an international consensus paper[19].

Cerebral SVD is a spectrum disorder, and is used to refer to the clinical, cognitive, neuroimaging and pathological findings thought to arise from diseased perforating cerebral arterioles[18]. SVD doubles the risk of future stroke[21]. However, SVD is also partly responsible for non-stroke brain diseases, such as contributing substantially to up to 45% of dementias[22], and can affect mood, gait and cause urinary problems[16,23–29]. Additionally, much of SVD is clinically silent and probably accumulates across the lifecourse before symptoms occur[18]. Observed pathological changes of SVD include vessel wall thickening that narrows the lumen (lipohyalinosis), hardening and loss of essential vessel wall elasticity (arteriosclerosis), inflammation or endothelial dysfunction and blood-brain barrier (BBB) dysfunction.

Table 1.1 MRI findings for lesions related to SVD

recent small subcortical infarct (RSSI)	"neuroimaging evidence of recent infarction in the territory of one perforating arteriole, with imaging features or clinical symptoms consistent with a lesion occurring in the previous few weeks"[19]
white matter hyperintensity (WMH) of presumed vascular origin	"WHM are hyperintense on T2-weighted sequences and can appear as isointense or hypointense (although not as hypointense as CSF) on T1-weighted sequences, depending on the sequence parameters and severity of the pathological change"[19]
lacune	"a round or ovoid, subcortical, fluid-filled (similar signal as CSF) cavity of between 3 mm and about 15 mm in diameter, consistent with a previous acute small deep brain infarct or haemorrhage in the territory of one perforating arteriole"[19]
perivascular space (PVS)	"fluid-filled spaces that follow the typical course of a vessel as it goes through grey or white mater. The spaces have signal intensity similar to that of CSF on all sequences. They appear linear when imaged parallel to the course of the vessel, and round or ovoid, with a diameter generally smaller than 3mm, when imaged perpendicular to the vessel"[19]
cerebral microbleed	"small (generally 2–5 mm in diameter, but up to 10 mm) areas of signal void with associated blooming seen on T2*-weighted MRI or other sequences that are sensitive to susceptibility effects. When imaged with T2*- weighted GRE sequences, cerebral microbleeds are well defined, of homogeneous low signal, and are either round or oval in shape"[19]

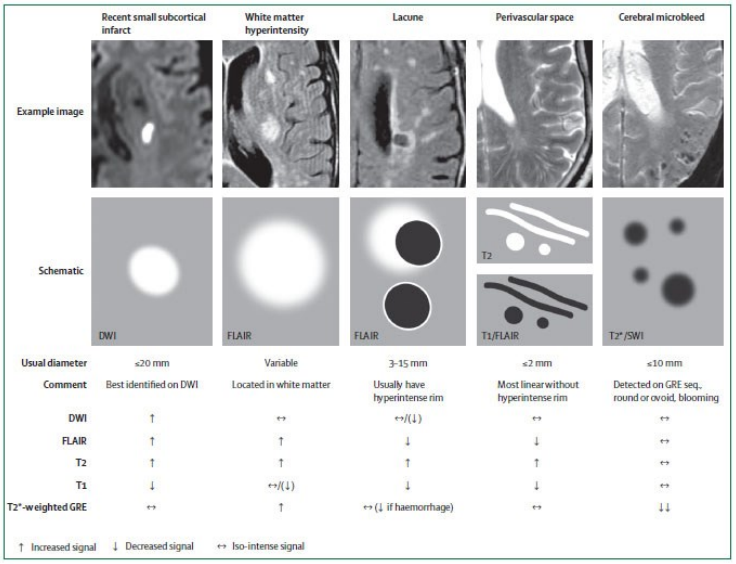


Figure 1.1 MRI findings for lesions related to SVD. Shows examples (upper row) and schemaic representation (middle row) of MRI features related to SVD, with a summary of imaging characteristics (lower row) for individidual lesions. DWI = diffusion-weighted imaging, FLAIR = fluid-attenuated inversion recovery, GRE = gradient-recalled echo, SWI = susceptability-weighted imaging. Reproduced from Wardlaw et al. *Lancet Neurol* 2013;12:822–38 with permission from the publishers Elsevier Ltd (license number 3832420235618 dated 19 March 2016).

What is the blood-brain barrier and why is it important?

The endothelium is a single cell lining of endothelial cells that covers the internal surface of the vascular system, including cerebral blood vessels where it is the BBB. The BBB forms a mechanical and functional barrier between the systemic circulation and brain tissue[30]. Junctional proteins link endothelial cells tightly to each other which forms a selectively permeable membrane which interfaces between circulating blood in the lumen and the vessel wall and interstitial spaces. The BBB has many physiological roles, including: selectively mediating the passage of molecules between blood and brain tissue; regulating inflammatory activity into and out of the brain; maintaining haemostasis; clotting; new blood vessel formation; and, via its production of nitric oxide, it has a critical role in the constriction and dilation of blood vessels. The BBB is an important function of the cerebrovascular endothelium and therefore endothelial dysfunction of various sorts may affect the BBB.

When the BBB becomes impaired (sometimes called BBB failure), blood-borne molecules may pass through the BBB, into the vessel wall and beyond, probably triggering an immune-mediated (inflammatory) reaction that damages neurons and glial cells (astrocytes, microglia and oligodendrocytes)[31]. Intravenous gadolinium given during MRI examinations has been shown to pass through the BBB more in lacunar versus non-lacunar stroke patients, and in older versus younger subjects, suggesting increased BBB permeability in these patients[32]. Leakage of plasma proteins through the BBB and into the vessel wall and surrounding brain tissue could be the first step in perivascular lesions and subsequent axonal damage[33].

Additionally, microbleeds (haemorrhagic leaks from small vessels) increase the risk of recurrent stroke (any stroke subtype) following ischaemic stroke[34], and this is consistent with BBB dysfunction and subsequent increased small vessel permeability. The permeability of the BBB increases with normal ageing but is accelerated in vascular or Alzheimer's dementia and with worsening SVD[35].

Cytokines – also known as signalling molecules – are small proteins released by cells to facilitate cell to cell communication during an immune response, and stimulate the movement of cells towards sites of inflammation, infection and trauma[36]. Recently, a novel technique to observe BBB dysfunction in response to the peripheral administration of lipopolysaccharide (i.e., a systemic inflammatory challenge) resulted in a rapid increase in the pro-inflammatory cytokine tumour necrosis factor alpha (TNF-α) in the serum and brain tissue of rats[37]. In mice there is worse ischaemic brain damage and more severe neurological deficits[38] when given a systemic inflammatory challenge versus controls, and the mechanism could be initiated by the expression of the cytokine interleukin-1 because the effect was exacerbated when interleukin-1 was administered, and attenuated with interleukin-1 receptor antagonist[38].

Cerebral inflammation after stroke may contribute to ischaemic damage. After stroke, endothelial cells express more intercellular adhesion molecule-1 (ICAM) to facilitate the transmigration of immune cells into the damaged brain tissue, and anti-ICAM antibodies have been shown to reduce brain injury in animal models[39] but these findings have not been translated for human use as a randomised, double-blinded, placebo-controlled trial (n=625 stroke patients) failed to show efficacy[40].

Under pathologic conditions a number of chemical mediators are released that increase BBB permeability and various mechanisms are implicated including hypoxia, infection, oxidative damage, activation of matrix metalloproteinases, inflammation and autoimmunity[30,41] but the precise steps are not yet fully understood. Increased cytokines result in greater endothelial adhesion and transmigration of immune cells across the BBB.

There are at least four different pathways by which peripheral inflammation communicates with the brain, including: by-pass of the BBB at the circumventricular organs (where there is a lack of a BBB); circulating cytokines may activate cerebral endothelial cells, which in turn activate perivascular macrophages that signal to the microglia within the parenchyma; cytokines may activate the vagus nerve, which communicates with the brainstem; and lastly cytokines may be actively transported by endothelial cells across the BBB[42].

The human immune system and inflammation

The human immune system is staggeringly complex. Maintenance of health and modulation of disease requires a swathe of interrelated mediating and signalling tasks, from pattern recognition of exogenous foreign material (bacteria, virus, unrecognised proteins, etc) or endogenous dangers such as dying cells, to tagging molecules for destruction, to destroying and clearing the offending material. There are two broad immune responses: innate (non-specific) and adaptive (specific). The innate response is the rapid, first-line defence but its lack of specificity means that it will attack and attempt to clear anything it does not recognise. In contrast, the adaptive immune response will selectively target pathogens which the immune system has been 'trained'

to recognise through prior exposure (e.g., CD8+ cells target virus infected cells, neutrophils target bacteria, etc).

The inflammatory hypothesis

Activation of the human immune system is sometimes generically described as "inflammation". A hallmark of inflammation is increased vascular permeability to facilitate the movement of immune response cells out of the blood and into the site of injury in order to deal with the injury. When the immune response continues in the absence of specific pathogenic material, the body can start to destroy its own tissues (autoimmunity) and then the protective immune response becomes self-harming, as in diseases like rheumatoid arthritis (RA), Crohn's and psoriasis. These diseases are characterised by non-resolving, systemic inflammation, and in the brain could influence the BBB. There is a broader literature on neuropsychiatric and neurological disorders with a primary inflammatory aetiology. Evidence is emerging that inflammation may play a role in the pathogenesis of Alzheimer's.

Inflammation plausibly explains some of the excess stroke risk due to atheromatous large vessel stroke as inflammation is involved in all stages of atherosclerosis from fatty streak formation to plaque disruption[43–45]. In sporadic SVD, inflammation and cell infiltrates are seen in the perforating arteriolar walls and microglial activation is seen in the perivascular tissue on pathology[46,47]. The source of the inflammation is not known, whether intrinsic or systemic, but is associated with raised plasma markers of inflammation in healthy older subjects[48]. C-reactive protein (CRP), one of the main clinical measures of inflammation, was associated with lacunar infarcts in a recent large (n=519) study, independent of age and traditional vascular risk factors[49]. Factors

that contribute to endothelial damage such as immune complex formation and complement activation/deposition might trigger vascular inflammation and disrupt small vessel integrity. In animal models (including animal models of arthritis) systemic inflammation induces brain inflammation[50,51] while inflammation is implicated as a pathological mechanism in dementia[52]. Systemic inflammation is associated with an increase in cognitive decline in Alzheimer's disease[53]. One group of patients that are known to have systemic inflammation are patients diagnosed with inflammatory rheumatic arthropathies, and we study such diseases in this thesis as an in vivo model of inflammation.

Inflammatory rheumatic diseases and increased stroke risk

Inflammatory rheumatic diseases are characterised by inflammation (signs include redness or heat, swelling and pain) and loss of function of one or more connecting or supporting structures (such as joints)[54]. Patients diagnosed with inflammatory rheumatic diseases such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) are at increased risk of cardiovascular disease including stroke in general (reviewed in Chapter 4)[55,56] but little is known about lacunar stroke or SVD in these patients. Many of the symptoms seen in SVD (failing cognition, changes in mood, gait disturbances) are also seen in patients with rheumatic diseases[57–60], and brain imaging biomarkers of SVD such as WMH are more common in these patients versus the general population in some[61] but not all[62] studies. Consequently, it is reasonable to hypothesise that the mechanisms that cause rheumatic symptoms could also trigger pre-clinical brain damage and increase lacunar stroke risk in these patients.

Biomarkers aid our understanding of disease mechanisms

Biomarkers provide insights into normal biological processes, pathogenic processes, or pharmacologic response to a therapeutic intervention[63]. Biomarkers are measured in blood (plasma or serum), cerebrospinal fluid and brain tissue and can be hypothesis-generating by providing mechanistic insights into underlying disease processes and pathways. Although observational studies are unable to determine causation, with accumulating evidence biomarkers become established and provide vital information on disease risk and prognosis (there were no randomised interventions testing the effects of smoking on lung cancer risk but evidence from observational studies became overwhelming). Other examples include:

- high serum cholesterol leads to the development of atherosclerosis[64] which is a major cause of large vessel stroke;
- elevated homocysteine (a breakdown product of methionine (found mostly in animal protein)) is an independent risk factor for Alzheimer's Disease[65,66].

Plasma biomarkers of inflammation, endothelial dysfunction and haemostasis may provide mechanistic insights to the cause of lacunar stroke and SVD via implicated processes such as systemic inflammation, BBB failure or occlusive microthrombus[18,67–71].

The common clinical marker of inflammation, CRP, was significantly raised in lacunar stroke (n=124) versus non-stroke controls (n=600), within 10 days of the stroke ($p<0.001$) and at three months follow-up ($p<0.001$)[72] but another study[73] did not find a difference in levels of CRP between lacunar stroke (n=128) and atherothrombotic stroke (n=151) or cardioembolic stroke (n=259) possibly indicating that inflammation is raised in all strokes.

A previous review[68] of symptomatic lacunar stroke versus non-stroke controls suggests a pathogenic role for endothelial dysfunction but this could simply reflect having an ischaemic stroke in general[70]. Another review[74] found CRP and homocysteine to differ significantly between ischaemic stroke and non-stroke controls, but did not assess levels of blood markers between ischaemic stroke subtypes.

Prior stroke studies often take the plasma samples too early making it difficult to isolate underlying immune system pathway activity independent from an acute phase response. Few studies have assessed a range of biomarkers simultaneously in one population. Studying biomarkers in SVD is one strategy for increasing our knowledge of SVD mechanisms, which is a necessary precursor to developing therapies to reduce the burden of SVD.

Summary and thesis aims

Stroke is a global health problem. Inflammation is implicated in large vessel stroke but the role of inflammation in SVD and lacunar stroke is less well understood. Inflammation in SVD is seen pathologically, but the source of the inflammation is not known. Therefore we undertook work to better understand inflammation in SVD, in particular the role of systemic inflammation, and our aims are split into three sections:

- **Section A.** To see if differences exist in plasma biomarkers of coagulation, fibrinolysis, endothelial dysfunction, and inflammation between lacunar stroke, other ischaemic stroke subtypes and non-stroke controls (by literature review (Chapter 2) and experiment (Chapter 3)) and to see if there are relationships between plasma biomarkers and common SVD features such as WMH (Chapter 3);
- **Section B.** To review associations between stroke, including stroke subtypes, and rheumatic diseases, and to determine if rheumatic diseases increase the risk of specific stroke subtypes or 'silent' vascular disease on neuroimaging (by literature review (Chapter 4) and via a large national data linkage study of a regional rheumatology service (Chapter 5));
- **Section C.** To conduct a pilot magnetic resonance (MR) neuroimaging study to assess the burden of SVD among patients with the inflammatory disease SLE versus a cohort of stroke patients (i.e., clinically overt SVD) and non-stroke controls, and to assess relationships between total SVD burden and biomarkers of inflammation and endothelial dysfunction (Chapter 7); and also investigate evidence of SVD mechanisms in the brain including microstructural damage (including a review of the literature) in SLE including relationships with cognition, fatigue, SLE disease activity and inflammatory markers (Chapter 8).

Chapter 2: Blood markers of coagulation, fibrinolysis, endothelial dysfunction, and inflammation in lacunar stroke versus non-lacunar stroke and non-stroke: Systematic review and meta-analysis

[now published: Wiseman et al. *Cerebrovasc Dis.* 2014;37:64–75]

Introduction

One-quarter of ischaemic strokes are lacunar in type making lacunar stroke an important stroke subtype. The aetiology of lacunar stroke is different from other stroke subtypes, usually being the symptomatic manifestation of SVD rather than large vessel atheroma or cardioembolism.

Plasma biomarkers could provide mechanistic insights. A previous review[68] of symptomatic lacunar stroke versus non-stroke controls suggests a pathogenic role for endothelial dysfunction but this could simply reflect having an ischaemic stroke in general[70]. Another review[74] found C-reactive protein (CRP), P-selectin and homocysteine to differ significantly between ischaemic stroke and non-stroke controls, but did not assess levels of blood markers between ischaemic stroke subtypes.

Our aims were to see if differences exist in blood markers between lacunar stroke and other ischaemic stroke subtypes by reviewing the literature for studies measuring coagulation, fibrinolysis, endothelial dysfunction and inflammation. To disentangle the acute phase response, the analysis was split on timing of the blood draw in relation to the stroke.

Methods

This review has been prepared in accordance with the *Preferred reporting items for systematic reviews and meta-analyses* (PRISMA) statement[75]. We extracted data and conducted the meta-analysis in accordance with *Meta-analysis of observational studies* (MOOSE) guidelines[76], modified using three reporting standards[77–79].

Search strategy

We used OVID to search MEDLINE (from 1966) and EMBASE (from 1980) on 15th July 2012 using a search strategy (Table 2.1) developed with advice from the Cochrane Stroke Group (http://stroke.cochrane.org/).

Table 2.1 Search strategy

1. brain ischemia/ or brain infarction/ or brain stem infarctions/ or cerebral infarction/ or hypoxia-ischemia, brain/ or stroke/
2. (isch?emi$ adj6 (stroke$ or apoplex$ or cerebral vasc$ or cerebrovasc$ or cva or attack$)).tw.
3. ((brain or cerebr$ or cerebell$ or vertebrobasil$ or hemispher$ or intracran$ or intracerebral or infratentorial or supratentorial or middle cerebr$ or mca$ or anterior circulation) adj5 (isch?emi$ or infarct$ or thrombo$ or emboli$ or occlus$ or hypoxi$)).tw.
4. 1 or 2 or 3
5. (lacun$ or small vessel$ or small infarct$ or microinfarct$ or subcortical lesion$ or subcortical infarct$ or microvascular$ or microcirculation$).tw.
6. 4 and 5
7. blood-brain barrier/ or endothel$, vascular/ or tunica intima/ or microcirculation/
8. (endotheli$ adj5 (function$ or dysfunction$ or impairment$)).tw.
9. (endogenous tissue plasminogen activator or endogenous tPA).tw
10. thrombosis.tw
11. fibrinogen.tw
12. fibrinolysis.tw
13. homocysteine.tw
14. (ICAM or Intra cellular adhesion molecule).tw
15. (VCAM or Vascular Cell Adhesion Molecule).tw
16. (IL6 or Interleukin 6).tw
17. (CRP or C reactive protein).tw
18. von Willebrand factor.tw
19. plasminogen activator inhibitor.tw
20. selectin$.tw
21. D-dimer.tw
22. (TNF or TNF-a or TNF-alpha or TNF-α or tumo?r necrosis factor alpha).tw
23. or/7-22
24. 6 and 23

Inclusion and exclusion

We included English language studies published in full measuring blood markers in plasma or serum as follows: tissue plasminogen activator (t-PA) as an indicator of fibrinolytic and thrombotic state; plasminogen activator inhibitor (PAI) as an inhibitor of t-PA; fibrinogen as a measure of coagulation; D-dimer as a measure of fibrinolysis; homocysteine as a marker of endothelial toxicity; von Willebrand factor (vWF) as a marker of endothelial damage; E-selectin, P-selectin, intercellular adhesion molecule-1 (ICAM) and vascular cellular adhesion molecule-1 (VCAM) as markers of endothelial activation and CRP, interleukin-6 (IL-6) and tumour necrosis factor alpha (TNF-α) as markers of inflammation. We excluded studies that did not provide information on blood markers in lacunar stroke and a control group.

Data extracted

Data was extracted on study population (sample size, age, sex, co-morbidities, current medications); study design; control group (including details on matching for age, sex and covariates); blinding of stroke assessor to blood markers and assay investigator to stroke diagnosis; blood markers assessed; time to all blood draws; whether the authors gave details on how samples were obtained, stored and processed and results of blood marker levels (both measures of average and spread where given). We recorded method of stroke diagnosis, criteria/system used for stroke subtyping, details of imaging, definition of lacunar stroke and the grade of clinician making the stroke diagnosis. We contacted one author[80] to clarify time of blood draw. Some studies drew blood at multiple time points. We recorded data at multiple time points where a relevant comparator was also available. We avoided duplicate publications.

Definition of lacunar stroke

Our gold standard definition of lacunar stroke was all of: reference to one of the classical clinical lacunar syndromes; no evidence of cortical dysfunction; imaging, including noting that a normal scan does not exclude a lacunar diagnosis; and mention of expertise of the person who subtyped the stroke. We relied on the clinical stroke definitions used in the primary papers but tried to harmonise these to a clinical stroke syndrome with support from imaging where available[19].

Meta-analysis

We used the Review Manager 5 software to calculate standardized mean differences (where data were in a suitable format) using the inverse variance method and a fixed effects model with 95% confidence interval (CI). Our forest plots compare lacunar stroke to non-lacunar stroke (atherothrombotic stroke and/or cardioembolic stroke) or to non-stroke. Studies[81–83] reporting a geometric mean with 95% CI were converted to standard deviations using methods described in the Cochrane Handbook[84].

Not all studies could be meta-analyzed due to heterogeneity of reporting. The following markers were reviewed solely by summary of individual study data: t-PA, PAI, E-selectin, P-selectin, ICAM, VCAM, CRP and TNF-α.

Where studies provided data at more than one acute time point, we meta-analysed only the first time point as this most often corresponded with data for non-stroke comparators. In order to see if an acute phase response affected the results we split our analysis into ‘acute’ and ‘chronic’ (bloods drawn up to and after 21 days of stroke respectively).

Results

We identified 1,468 full papers. In all, 1,389 titles were excluded following a survey of titles and abstracts leaving 79 for reading. Of these 10 were excluded (unable to translate) and 32 did not meet inclusion criteria (duplicates; asymptomatic subjects; non-relevant blood markers; no control group). A hand-search found five papers so 42 papers were eligible for inclusion (Figure 2.1), including 4,816 ischaemic strokes, of which 2,196 were lacunar, and 2,500 non-stroke controls.

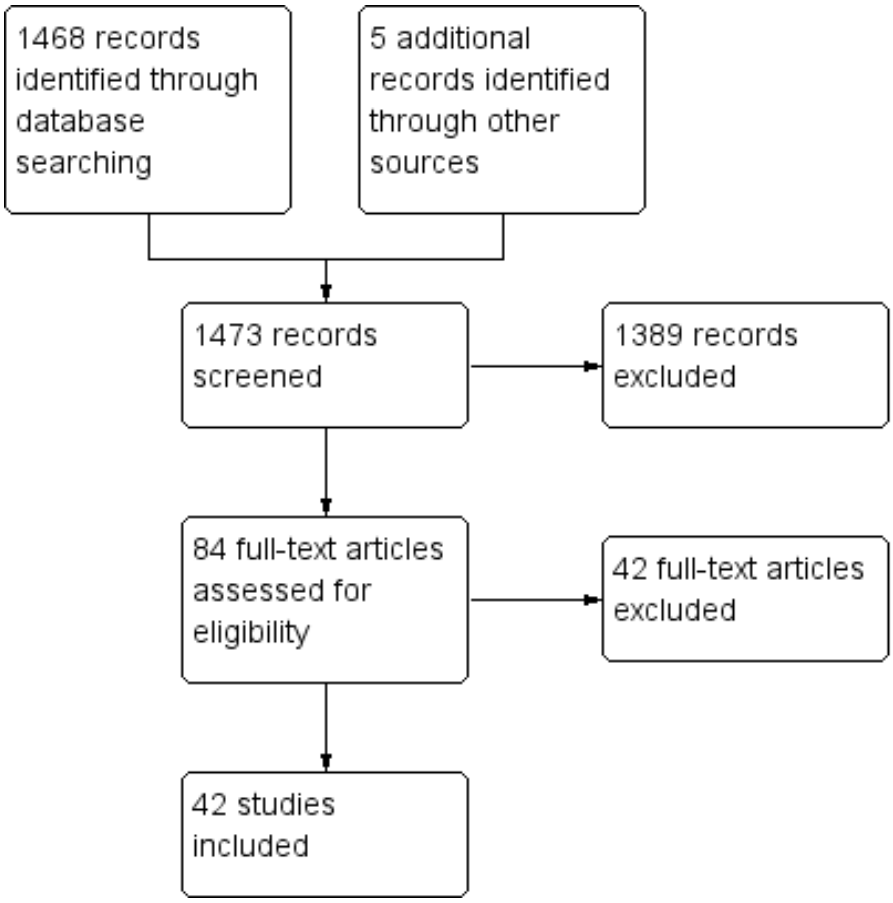


Figure 2.1 Summary of search and selection

Critical appraisal of included studies

Over 50% of studies (22/42) used Trial of Org 10172 in Acute Stroke Treatment (TOAST)[85] to subtype ischaemic stroke. Just four studies[81,86–88] (under 10%) met our gold standard definition of lacunar stroke. More than half of the studies (57%) reported a 'minimal' definition of lacunar stroke. One study[89] failed to define lacunar stroke. Most studies (31/42) recruited patients consecutively. One study recruited non-consecutively[86] and 10/42 did not report on recruitment. Two-thirds of studies reported excluding cases based on co-existing disease such as infection, cancer, inflammatory disease and renal failure. One-third of studies did not report on exclusion criteria. Most studies (24/42) reported matching controls by age and sex. Three studies did not report on matching. Matching for co-morbidities varied from study to study. Under 20% of studies (8/42) reported blinding of stroke assessor to blood marker values. Fewer still reported blinding of laboratory staff to stroke data. Characteristics of included studies and quality of assessment thereon is given in Tables 2.2 and 2.3, respectively.

Table 2.2 Characteristics of included studies

Study	Markers	Imaging	Stroke subtyping	Number of subjects / of which lacunar	Age of subjects, years (SD or range)
Takano, 1992	D-dimer	CT and angio	Clinical + imaging	73 / 23	65
Kilpatrick, 1993	Fibrinogen	CT in all, Doppler / angio in some	Clinical + imaging	19 / 9	69.7 (12.8)
Beamer, 1995	IL-6	CT or MR	NSt	50 / 23	66 (9)
Lindgren, 1996	t-PA, PAI	CT	OCSP	135 / 33	75.2 (38-98)
Bath, 1998	vWF, P-selectin, fibrinogen	CT	OCSP	163 / 56	74 (7)
Eikelboom, 2000	Homocysteine	CT in all, Doppler in some	OCSP	219 / 68	66.1 (12.4)
Kataoka, 2000	Fibrinogen, D-dimer	CT or MR, angio	NINDS III	137 / 58	67.6 (12.6)
Vila, 2000	IL-6	CT	TOAST	231 / 33	NSt
Ageno, 2002	D-dimer	CT and Doppler	OCSP	126 / 31	74.7
Castellanos, 2002	ICAM-1, IL-6, TNF-α	CT or MR and Doppler	OCSP	113 / 113	69.7 (9.3)
Kozuka, 2002	vWF, E-selectin, P-selectin,	CT or MR	NINDS III	52 / 27	68 (63-72)
Salobir, 2002	t-PA, PAI, fibrinogen, D-dimer	CT	OCSP	192 / 16	42 (36-48)
Hassan, 2003	ICAM	Neuroimaging and Doppler or MRA	OCSP	110 / 110	67.1 (10.3)
Hassan, 2004	Homocysteine	Neuroimaging and Doppler or MRA	OCSP	172 / 172	67.1 (10.3)
Parnetti, 2004	Homocysteine	NSt	TOAST	161 / 50	72 (13)
Salobir, 2004	IL-6	CT	OCSP	192 / 16	42 (36-48)
Jood, 2005	t-PA, PAI	CT or MR, Doppler / angio in some	TOAST	600 / 124	58 (7)
Licata, 2006	TNF-α	CT or MR or ce-CT	TOAST	60 / 29	72 (64-82)
Ladenvall, 2006	CRP	CT or MR, Doppler / angio in some	TOAST	600 / 124	58 (7)
Domac, 2007	IL-6, TNF-α	CT or MR and Doppler	OCSP	70 / 19	68.5 (12.4)
Khan, 2007	Homocysteine	CT or MR and Doppler	TOAST	47 / 47	65.3 (10.4)
Guldiken, 2008	IL-6	CT	TOAST	28 / 16	66.9 (9.8)
Jood, 2008	Fibrinogen	CT or MR, Doppler / angio in some	TOAST	599 / 123	58 (7)
Khan, 2008	Homocysteine	CT or MR, Doppler / angio in most	Mod TOAST	457 / 152	65.4 (12.2)
Montaner, 2008	CRP, D-dimer	Neuroimaging and carotid ultrasound	TOAST	707 / 128	72 (12)
Nakase, 2008	CRP, IL-6, TNF-α	NSt	TOAST	105 / 42	71.4 (13.8)
Yokokawa, 2008	PAI, CRP, homocysteine	MR	Clinical + imaging	222 / 55	71 (41-88)
Bronus, 2009	D-dimer	CT or MR, Doppler / angio	TOAST	128 / 23	72.2 (11.7)
Licata, 2009	vWF, CRP, IL-6, TNF-α	CT or MR or ce-CT	TOAST	120 / 46	72 (64-82)
Tsai, 2009	P-selectin	MR and MRA	TOAST	54 / 32	68.1 (10.2)
Tuttolomondo, 2009a	t-PA, vWF, ICAM, VCAM, PAI, E-selectin, P-selectin, IL-6, TNF-α	CT or MR	TOAST	107 / 32	71 (63-80.5)
Tuttolomondo, 2009b	t-PA, ICAM, VCAM, PAI, E-selectin, P-selectin	CT or MR or ce-CT	TOAST	120 / 46	72 (64-82)
Ilhan, 2010	PAI, P-selectin, D-dimer	CT or MR, Doppler / angio	TOAST	30 / 30	65.4 (10.8)
Isenegger, 2010	D-dimer	CT or MR, Doppler / angio	TOAST	98 / 5	67 (19)
Alvarez-Perez, 2011	Fibrinogen, CRP, D-dimer	Doppler	TOAST	200 / 50	72 (11.5)
Beer, 2011	vWF, E-selectin, Fibrinogen, CRP, homocysteine	NSt	TOAST	129 / 25	66.1 (12.7)
Hanson, 2011	vWF	CT or MR, Doppler / angio	TOAST	599 / 123	58 (7)
Jeong, 2011	Homocysteine	MR and Doppler	TOAST	83 / 83	62.1 (11.9)
Pavlovic, 2011	Homocysteine	MR and Doppler	NSt	95 / 95	59.8 (10.9)
Supanc, 2011	ICAM, VCAM	CT and Doppler	Neurologist	110 / 43	70.2 (9.6)
Turgut, 2011	P-selectin, CRP	CT or MR, Doppler / angio	TOAST	72 / 20	65.2 (1.4)
Zhang, 2011	Fibrinogen	CT or MR	Clinical + imaging	626 / 262	40.9 (5.4)

CRP = C-reactive protein. CT = computed tomography. ICAM = intercellular adhesion molecule-1. IL-6 = interleukin-6. MR = magnetic resonance. NSt = not stated. OCSP = Oxfordshire Community Stroke Project. TNF-α = tumour necrosis factor alpha. TOAST = Trial of Org 10172 in Acute Stroke Treatment. t-PA = tissue plasminogen activator. VACM = vascular cellular adhesion molecule-1. vWF = von Willebrand factor.

Table 2.3 Quality assessment of included studies

Study	Quality of definition of lacunar stroke*	Cases consecutively recruited?	Vascular risk factor - history taken?	Cases excluded if co-existing inflammatory disease, infection, cancer or renal failure?	Controls matched for age and sex	Controls matched for co-morbidities	Stroke assessor blinded to plasma marker	Plasma marker intra assay variability reported?
Takano, 1992	1	Yes	Yes	Yes	Age only	NSt	NSt	NSt
Kilpatrick, 1993	4	No	Yes	Yes	e	e	Yes	f
Beamer, 1995	0	NSt	Yes	Yes [a]	Age only	NSt	NSt	Yes
Lindgren, 1996	2	Yes	Yes	NSt	Sex only	Partly [q]	NSt	Yes
Bath, 1998	1	Yes	NSt	NSt	Yes	NSt	NSt	Yes
Eikelboom, 2000	4	Yes	Yes	NSt	Yes	Yes	Yes	NSt
Kataoka, 2000	2	NSt	NSt	Yes	Yes	Partly [p]	NSt	Yes
Vila, 2000	1	Yes	NSt	Yes	NSt	NSt	NSt	NSt
Ageno, 2002	1	NSt	NSt	Yes	Age only	NSt	NSt	Yes
Castellanos, 2002	4	Yes	Yes	Yes	NSt	NSt	Yes	NSt
Kozuka, 2002	1	Yes	Yes	Yes	Age only	NSt	NSt	Yes
Salobir, 2002	1	NSt	NSt	NSt	Yes	Yes	NSt	NSt
Hassan, 2003	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Hassan, 2004	3	Yes	Yes	NSt	Yes	Partly [o]	Yes	Yes
Parnetti, 2004	1	Yes	Yes	Yes	Yes	Partly [n]	NSt	Yes
Salobir, 2004	1	Yes	Yes	NSt	Yes	Partly [m]	NSt	NSt
Jood, 2005	1	Yes	Yes	NSt	Yes	Yes	NSt	Yes
Licata, 2006	1	Yes	Yes	Yes	Yes	Partly [b]	NSt	NSt
Ladenvall, 2006	1	Yes	Yes	NSt	Yes	Yes	NSt	Yes
Domac, 2007	4	Yes	NSt	Yes	Age only	NSt	Yes	NSt
Khan, 2007	1	NSt	Yes	NSt	Yes	Yes	NSt	NSt
Guldiken, 2008	3	NSt	Yes	Yes	Yes	Partly [l]	NSt	Yes
Jood, 2008	1	Yes	Yes	NSt	Yes	Yes	NSt	Yes
Khan, 2008	3	Yes	Yes	Yes [j]	Yes	Partly [k]	NSt	NSt
Montaner, 2008	3	Yes	Yes	Yes	e	e	Yes	NSt [i]
Nakase, 2008	1	Yes	Yes	Yes	e	e	NSt	NSt
Yokokawa, 2008	1	NSt	Yes	NSt	e	e	NSt	NSt
Bronus, 2009	2	Yes	Yes	Yes	e	e	NSt	f
Licata, 2009	1	Yes	Yes	Yes	Yes	Yes	NSt	Yes
Tsai, 2009	2	Yes	Yes	Yes	Yes	Yes	NSt	NSt
Tuttolomondo, 2009a	3	NSt	Yes	Yes	Yes	Yes	NSt	Yes
Tuttolomondo, 2009b	1	Yes	Yes	Yes	Yes	Yes	NSt	Yes
Ilhan, 2010	3	NSt	NSt	Yes [h]	NSt	NSt	NSt	NSt
Isenegger, 2010	1	Yes	Yes	Yes	e	e	Yes	NSt
Alvarez-Perez, 2011	1	Yes	Yes	Yes	Yes	Partly [g]	NSt	f
Beer, 2011	1	NSt	NSt	Yes [d]	e	e	NSt	NSt
Hanson, 2011	1	Yes	Yes	NSt	Yes	Yes	NSt	Yes
Jeong, 2011	2	Yes	Yes	NSt	Yes	Partly [r]	NSt	Yes
Pavlovic, 2011	1	Yes	Yes	Yes [c]	Yes	NSt	NSt	NSt
Supanc, 2011	1	Yes	NSt	Yes	Yes	NSt	NSt	NSt
Turgut, 2011	1	Yes	Yes	Yes	Age only	Yes	NSt	NSt
Zhang, 2011	3	Yes	Yes	NSt	e	e	NSt	NSt

No = used when authors specifically report in the negative, otherwise NSt = not stated. a. Although infection not an exclusion criteria. b. Not matched for blood pressure or cholesterol. c. Excluded if renal insufficiency, B12 and folate deficiency and alcohol abuse. d. Excluded if high glucose or co-morbid acute illness. e. No non-stroke control group, study investigates differences in biomarkers between subtypes of ischaemic stroke only. f. Laboratory normal ranges reported. g. Not matched for hypertension. h. Excluded if ICH, cardiac sources of emboli or concomitant severe systemic disease. i. Authors report testing samples twice but do not report intra-assay variability statistic. j. Excluded if on folic acid or Vit B12 due to the influence on homocysteine. k. Not matched for hypertension and diabetes. l. Not matched for systolic blood pressure and cholesterol. m. Not matched for hyperlipidemia. n. Not matched for hyperlipidemia. o. Not matched for hypertension, smoking and diabetes. p. Hypertension in atherothrombotic and lacunar subtypes not matched to controls. q. Not matched for diabetes and ischaemic heart disease. r. Not matched for hypertension. * We assessed the quality of the definition of lacunar stroke as follows: 0 = **no definition**, 1 = **minimal** (such as imaging + reference to recognized ischaemic stroke subtyping system), 2 = **acceptable** (such as imaging + reference to a recognized lacunar syndrome clinically), 3 = **full** (such as imaging including noting that a normal scan does not exclude a lacunar diagnosis + reference to one of the classical lacunar syndromes + mention of no evidence of cortical dysfunction, and 4 = **gold standard** as 3 but also reported on who subtyped the stroke.

Plasma biomarkers

Plasma biomarker data of included studies is reported here (see also Table 2.4).

Coagulation / fibrinolysis

t-PA: Five studies[90–94] (251 lacunar strokes) of which one study[92] contributes 50% of the data. There were insufficient data in an appropriate format for meta-analysis. Individual studies suggest t-PA was significantly higher in lacunar stroke versus non-stroke controls, both acutely and chronically. Meanwhile, t-PA does not differ between lacunar stroke and other stroke subtypes, either acutely or chronically.

PAI: Seven studies[90–96] (336 lacunar strokes) but available data did not permit meta-analysis. Jood et al.[92] report levels of PAI significantly higher in lacunar stroke versus non-stroke controls acutely and chronically. They also report lower PAI in lacunar stroke versus atherothrombotic and cardioembolic stroke acutely but not chronically[92]. Four other studies report no difference between lacunar and non-lacunar stroke[90,93–95].

Fibrinogen: Nine studies[86,89,91,97–102] (622 lacunar strokes), of which six permitted meta-analysis (Figure 2.2). Fibrinogen showed no difference in lacunar stroke versus non-stroke controls acutely but was significantly higher in lacunar stroke chronically. Fibrinogen was significantly lower in lacunar stroke versus other ischaemic stroke subtypes, both acutely (atherothrombotic SMD -0.37 (95% CI -0.51 to -0.22); cardioembolic SMD -0.83 (-1.15 to -0.51)) and chronically (Figure 2.2), although the chronic data comprised only one study[98]. Two[97,100] of three[97,99,100] studies that could not be meta-analyzed report no difference in levels of fibrinogen between lacunar stroke and non-lacunar stroke in the acute phase.

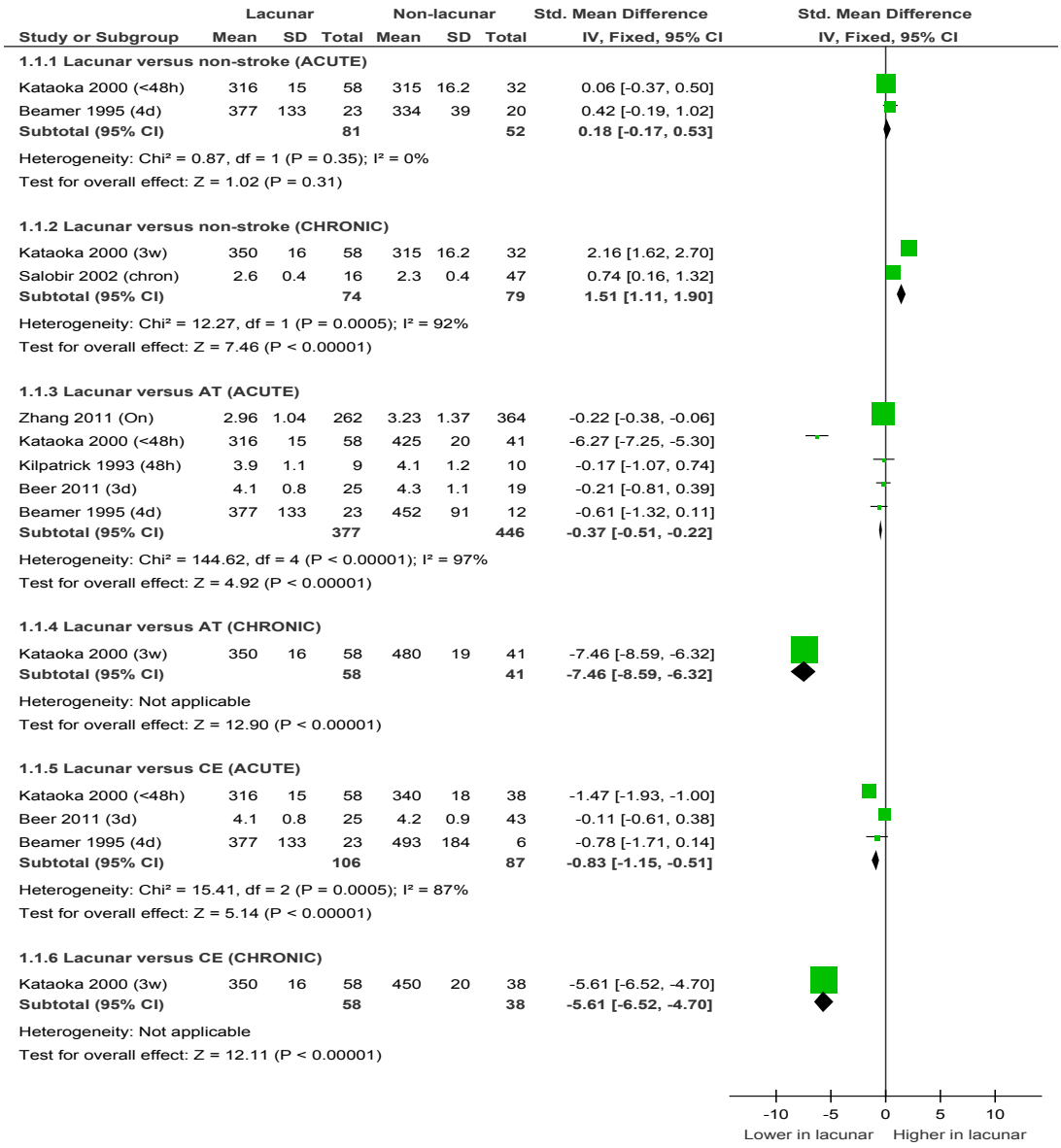


Figure 2.2 Forest plot – fibrinogen. Standardized mean difference of blood markers in lacunar stoke versus non-stroke controls and versus non-lacunar stroke at different times after stroke. AT = atherothrombotic, CE = cardioembolic

D-dimer: Nine studies[73,91,96,98,100,103–106] (364 lacunar strokes) of which four could be meta-analyzed (Figure 2.3). D-dimer was significantly higher in lacunar stroke versus non-stroke controls, both acutely (SMD 1.42 (1.14 to 1.69)) and chronically (SMD 3.22 (2.65 to 3.78)). D-dimer was significantly lower in lacunar versus non-lacunar

stroke, both atherothrombotic (e.g. acute SMD -3.59 (-4.06 to -3.12)) and cardioembolic (e.g. acute SMD -5.73 (-6.38 to -5.09)), acutely and chronically. The largest (n=128 lacunar strokes) of five studies[73] that could not be meta-analyzed found no difference in D-dimer between lacunar and atherothrombotic stroke acutely (which disagrees with the meta-analysis) and found D-dimer significantly lower in lacunar versus cardioembolic stroke acutely (in agreement with the meta-analysis).

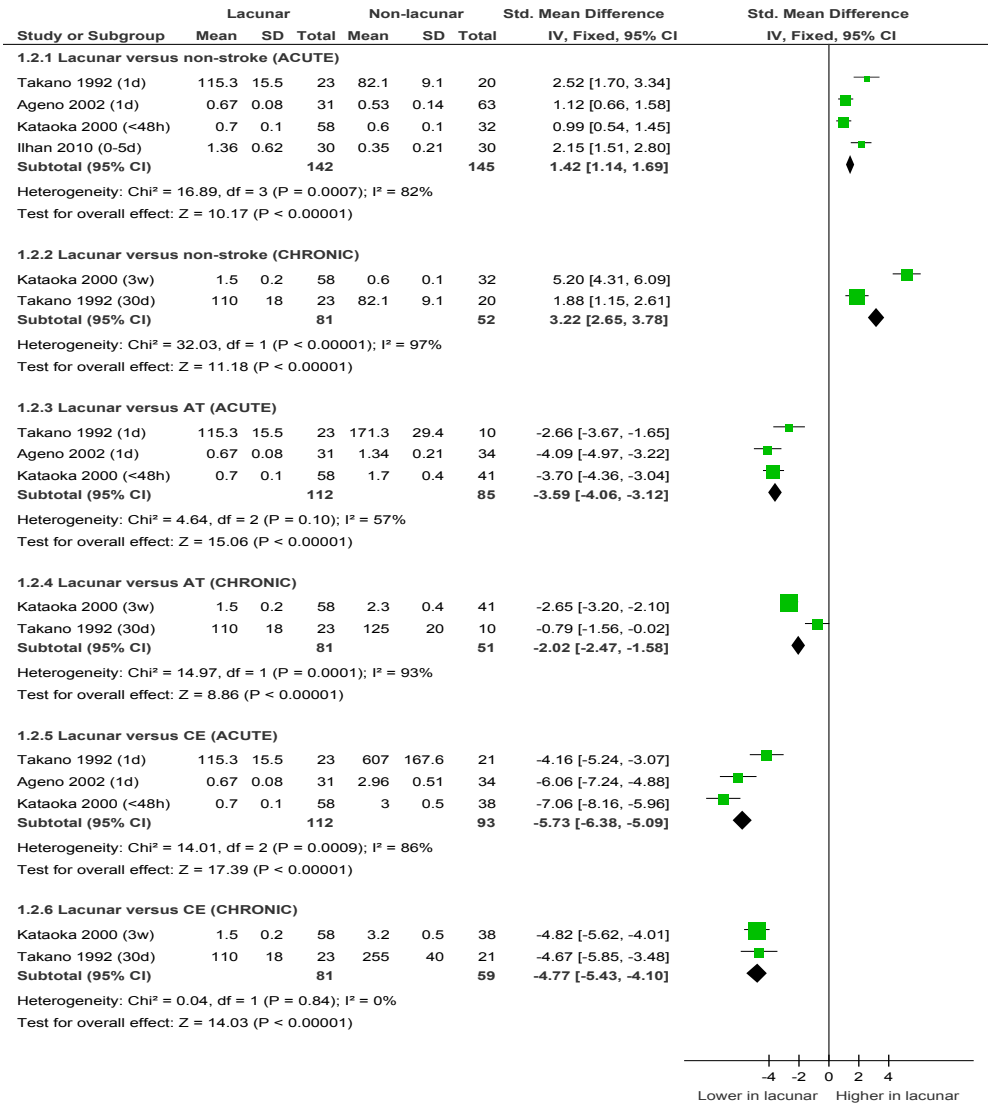


Figure 2.3 Forest plot – D-dimer. Standardized mean difference of blood markers in lacunar stroke versus non-stroke controls and versus non-lacunar stroke at different times after stroke. AT = atherothrombotic, CE = cardioembolic

Endothelial activation / dysfunction

Homocysteine: Nine studies[80–82,95,101,107–110] (747 lacunar strokes) of which eight could be meta-analyzed (Figure 2.4). Homocysteine was significantly higher in lacunar stroke versus non-stroke controls, both acutely (e.g. SMD 0.55 (0.42 to 0.69)) and chronically. Studies comparing lacunar to non-lacunar stroke drew blood acutely only and found no difference (atherothrombotic SMD -0.02 (-0.22 to 0.18); cardioembolic SMD 0.16 (-0.02 to 0.35)). One study[95] not included in the meta-analysis found no difference in homocysteine between lacunar and non-lacunar stroke acutely.

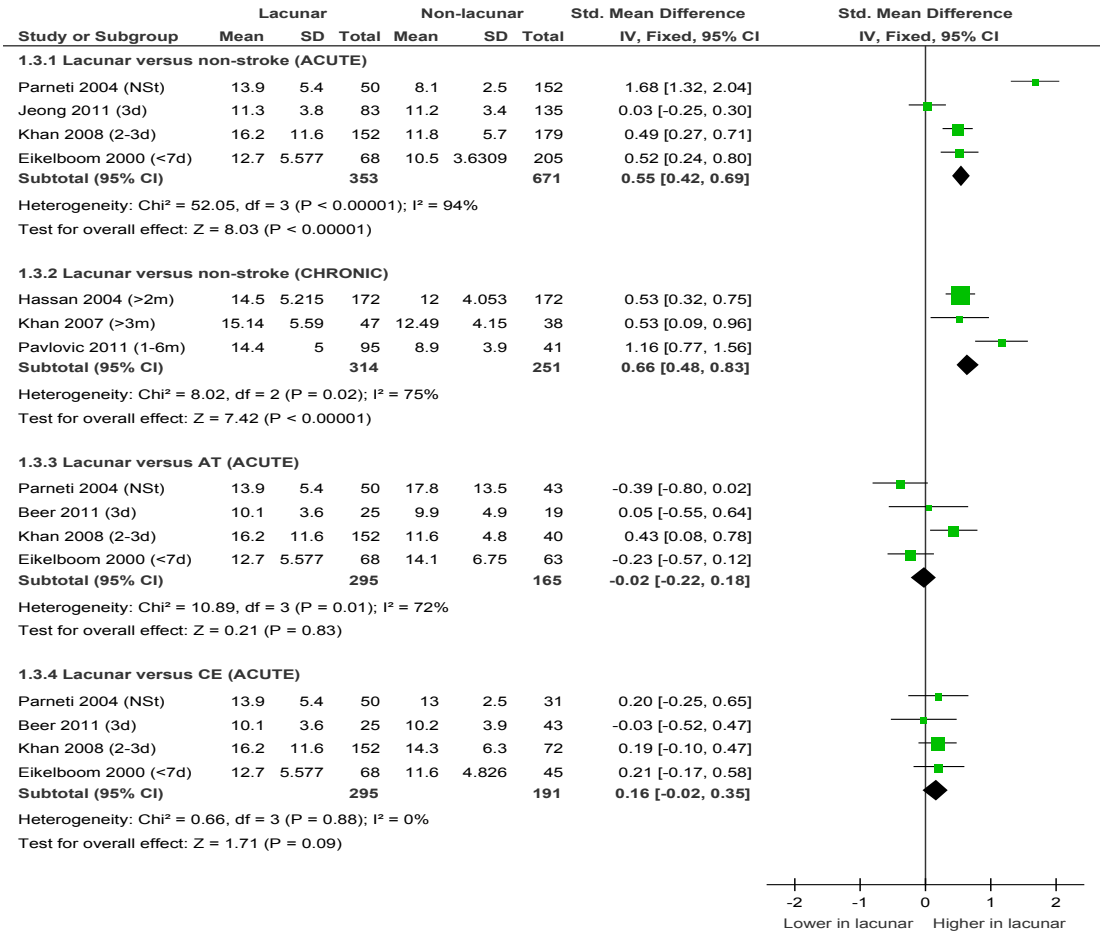


Figure 2.4 Forest plot – homocysteine. Standardized mean difference of blood markers in lacunar stoke versus non-stroke controls and versus non-lacunar stroke at different times after stroke. AT = atherothrombotic, CE = cardioembolic

vWF: Six studies[83,93,97,101,111,112] (293 lacunar strokes) of which two[83,101] were meta-analyzable (Figure 2.5). vWF was significantly higher in lacunar stroke versus non-stroke controls acutely. vWF was significantly lower in lacunar versus non-lacunar stroke (both atherothrombotic (SMD -0.34 (-0.61 to -0.08)) and cardioembolic (SMD -0.38 (-0.62 to -0.14)) acutely. The four non meta-analyzable[93,97,111,112] studies show conflicting results.

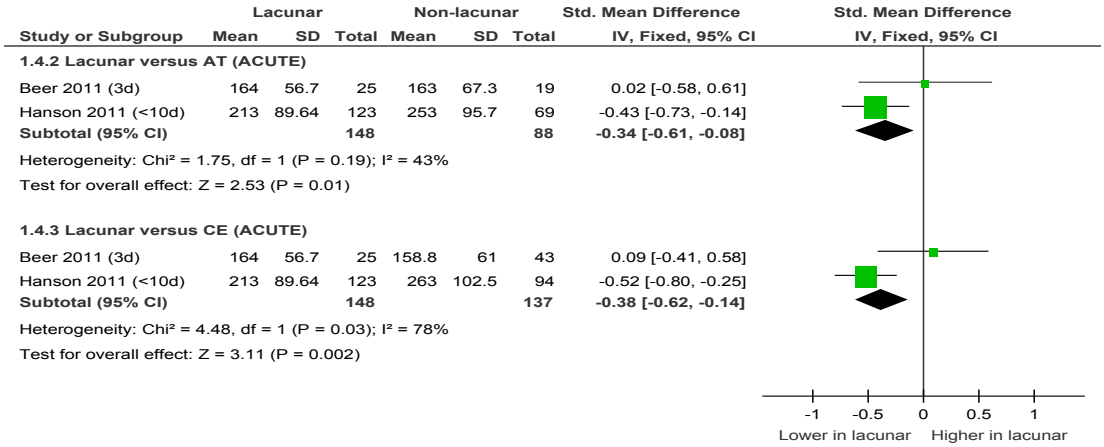


Figure 2.5 Forest plot – von Willebrand factor. Standardized mean difference of blood markers in lacunar stoke versus non-stroke controls and versus non-lacunar stroke at different times after stroke. AT = atherothrombotic, CE = cardioembolic

E-selectin: Four studies[93,94,101,111] (130 lacunar strokes) but available data did not permit meta-analysis. One study[111] found E-selectin significantly higher in lacunar stroke versus non-stroke controls acutely but not at one month. Individual studies' reports suggest no difference between lacunar and non-lacunar stroke.

P-selectin: Seven studies[93,94,96,97,111,113,114] (227 lacunar strokes) but available data did not permit meta-analysis. P-selectin was significantly higher in lacunar versus non-stroke acutely in two studies[111,114] but another[96] found no difference. One study[113] found P-selectin significantly lower in lacunar versus atherothrombotic stroke acutely

but all other studies found no difference. Tsai and colleagues[113] continued to show levels to be significantly lower at one month but this difference disappeared at three months. Only one study[97] found P-selectin significantly lower in lacunar versus cardioembolic stroke acutely; all other studies found no difference or did not report significance levels.

ICAM: Five studies[87,93,94,115,116] (344 lacunar strokes) but available data did not permit meta-analysis. One study[87] found significantly higher ICAM in lacunar stroke versus non-stroke controls acutely; another study[115] found ICAM significantly higher in lacunar versus non-stroke controls chronically. There was no difference between lacunar and other stroke subtypes acutely and no data for lacunar versus non-lacunar stroke chronically.

VCAM: Three studies[93,94,116] (121 lacunar strokes) but available data did not permit meta-analysis. Results suggested no difference between lacunar stroke and non-stroke acutely and no difference between lacunar and non-lacunar stroke acutely. No studies measured VCAM chronically.

Inflammation

CRP: Eight studies[72,73,95,100,101,112,114,117] (490 lacunar strokes) but available data did not permit meta-analysis. Two studies[72,73] provide just over 50% of the data. CRP was higher in lacunar stroke versus non-stroke acutely in three studies[72,100,114], significantly so in two and a third[100] that did not report if the higher value was significant. CRP was significantly lower in lacunar versus atherothrombotic stroke in one study[100] acutely; all other studies report no difference (or did not state significance) between lacunar and non-lacunar stroke, acutely and chronically.

TNF-α: Five studies[87,88,93,112,117] (252 lacunar strokes) but available data did not permit meta-analysis. One study[87] provides 45% of the data. Two studies[87,88] found levels of TNF-α significantly higher in lacunar stroke versus non-stroke controls acutely. TNF-α was significantly lower in two[93,112] and no different in two[88,117] studies reporting on lacunar versus non-lacunar stroke acutely. No studies measured TNF-α chronically.

IL-6: Nine studies[87–89,93,112,117–120] (340 lacunar strokes) of which five could be meta-analyzed (Figure 2.6). IL-6 was significantly higher in lacunar stroke versus non-stroke acutely (SMD 1.3 (0.9, 1.7)). IL-6 was significantly lower in lacunar versus non-lacunar stroke: both atherothrombotic (SMD -0.37 (-0.63 to -0.10)) and cardioembolic (SMD -0.52 (-0.82 to -0.22)) acutely. One non-metaanalyzable study[87] found IL-6 significantly higher in lacunar stroke versus non-stroke acutely while two non-metaanalyzable studies[93,112] found IL-6 significantly lower in lacunar versus non-lacunar stroke acutely, all in agreement with the meta-analyzed studies.

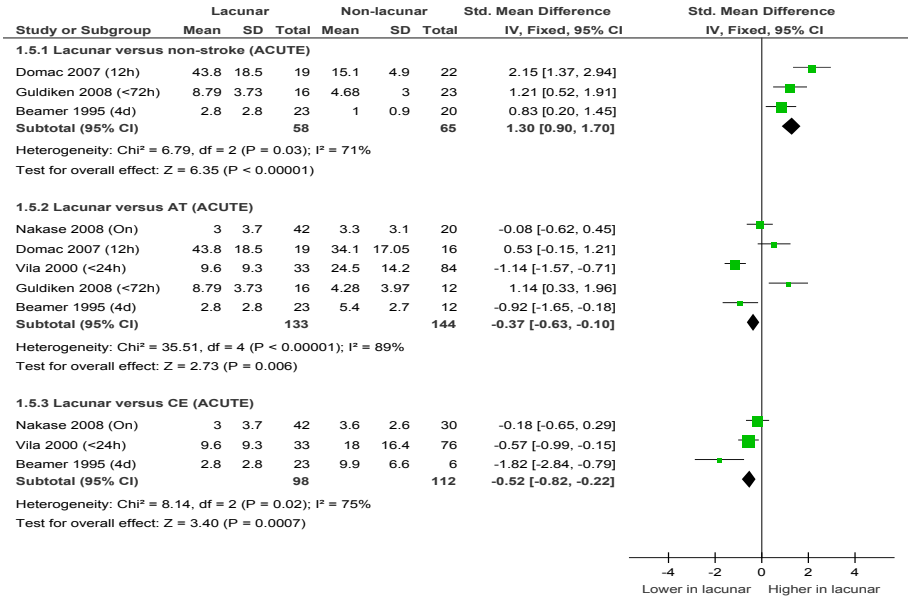


Figure 2.6 Forest plot – Il-6. Standardized mean difference of blood markers in lacunar stoke versus non-stroke controls and versus non-lacunar stroke at different times after stroke. AT = atherothrombotic, CE = cardioembolic

Table 2.4 Results from included studies

					Lacunar versus:							
	Units	**Time**	**n**	**Lacunar**	**n**	**Non stroke**	**p1**	**n**	**AT**	**p2**	**n**	**CE**	**p3**
t-PA													
Lindgren, 1996	ug/l	2d	33	11	77	6	Sig1	34	9.5 ♣	≠1	38	9 ♥	≠1
Salobir, 2002	ng/ml	chron	16	6.9 (5.8-8.5)	47	5.3 (4.1-7.1)	p<0.05	--	--	--	--	--	--
Jood, 2005	ug/l	<10d	124	11.8 (8.4-14.7)	600	9.8	p<0.001	73	12.6 (9.7-17.5)	NSt	98	14.1 (10.2-18.7)	NSt
		3m	124	11.2 (8.9-13.2)	--	Used <10d data	p<0.001	73	12.5 (9.7-16.3)	NSt	98	13.2 (9.5-16.3)	NSt
Tuttolomondo, 2009a	pg/ml	<12h	32	27 (20-39)	102	55 (29-88)	§	41	34 (14-43)	p=0.85^	29	21 (17-23)	p=0.85^
Tuttolomondo, 2009b	pg/ml	<72h	46	28 (20-38)	123	74 (59-98)	§	50	24 (14-36)	p=0.85^	20	22 (17-23)	p=0.85^
PAI													
Lindgren, 1996	ug/l	2d	33	22	77	8	Sig2	34	15 ♣	≠1	39	12 ♥	≠1
Salobir, 2002	ng/ml	chron	16	10.6 (5.9-16.4)	47	8.8 (6.0-16.1)	≠	--	--	--	--	--	--
Jood, 2005	ug/l	<10d	124	51.2 (38.7-71.8)	600	39.1 (23.6-60.0)	p<0.001	73	56.7 (38.8-74.2)	NSt	98	52.7 (35-68.8)	NSt
		3m	124	53.8 (34.4-77.1)	--	Used <10d data	p<0.001	73	61.9 (44.1-78.8)	≠	98	52.9 (34.2-75.7)	≠
Yokokawa, 2008	ng/ml	On	55	19 (10-94)	--	--	--	87	19 (10-200)	≠	19	25 (10-62)	≠
Tuttolomondo, 2009a	pg/ml	<12h	32	145 (118-192)	102	23 (11-24)	§	41	124 (89-159)	p=0.41^	29	152 (103-197)	p=0.41^
Tuttolomondo, 2009b	pg/ml	<72h	46	155 (108-172)	123	21 (12-26)	§	50	134 (98-168)	p=0.61^	20	162 (123-187)	p=0.61^
Ilhan, 2010	ng/ml	0-5d	30	50.74 ±10.49	30	57.14 ±9.72	p=0.034	--	--	--	--	--	--
		15d	30	48.90 ±8.95	--	--	--	--	--	--	--	--	--
		30d	30	51.75 ±12.39	--	--	--	--	--	--	--	--	--
D-dimer													
Takano, 1992	ng/ml	1d	23	115.3 ±15.5	20	82.1 ±9.1	≠	10	171.3 ±29.4	NSt	21	607 ±167.6	NSt
		7d	23	125 ±20 ♠	--	--	≠2	10	280 ±35 ♠	NSt	21	400 ±80 ♠	NSt
		30d	23	110 ±18 ♠	--	--	≠2	10	125 ±20 ♠	NSt	21	255 ±40 ♠	NSt
Kataoka, 2000	ng/ml	<48h	58	0.7 ±0.1 ♠	32	0.6 ±0.1	≠	41	1.7 ±0.4 ♠	NSt	38	3.0 ±0.5 ♠	p<0.001
		1w	58	1.4 ±0.2 ♠	--	--	≠	41	2.2 ±0.4 ♠	NSt	38	5.2 ±0.8 ♠	p<0.0001
		3w	58	1.5 ±0.2 ♠	--	--	NSt	41	2.3 ±0.4 ♠	NSt	38	3.2 ±0.5 ♠	p<0.0001
Ageno, 2002	ug/ml	1d	31	0.67 ±0.08	63	0.53 ±0.14	≠	34	1.34 ±0.21	p=0.01	34	2.96 ±0.51	p=0.009
		6d	31	0.72 ±0.06	--	--	--	34	1.53 ±0.26	P=0.002	34	2.58 ±0.4	p=0.009
		12d	31	0.64 ±0.1	--	--	--	34	2.91 ±0.59	p=0.03	34	3.79 ±0.53	p=0.009
Salobir, 2002	ng/ml	chron	16	57 (54-83)	47	59 (50-73)	≠	--	--	--	--	--	--
Montaner, 2008	ug/ml	8h	128	0.6 (0.3-1.3)	--	--	--	151	0.5 (0.2-1.7)	≠	259	1.1 (0.5-2.3)	p<0.001
Brouns, 2009	ng/ml	<9h	23	362	--	--	--	105	1500 ‡	p<0.001	--	--	--
Ilhan, 2010	ug/dl	0-5d	30	1.36 ±0.62	30	0.35 ±0.21	p<0.001	--	--	--	--	--	--
		15d	30	0.77 ±0.24	--	--	--	--	--	--	--	--	--
		30d	30	0.64 ±0.17	--	--	--	--	--	--	--	--	--
Isenegger, 2010	ug/l	4h	5	290 (217)	--	--	--	11	465 (835)	NSt	53	615 (405)	p<0.001 &
Alvarez-Perez, 2011	ng/ml	<48h	50	224.5 (162-281)	50	190.5 (140-240.5)	p=0.042	50	218 (156.8-406)	NSt	50	500 (235.5-675)	p<0.0001
Homocysteine													
Eikelboom, 2000	umol/l	<7d	68	12.7 (11.4-14.1) ♂	205	10.5 (10-11) ♂	p=0.004	63	14.1 (12.5-15.9) ♂	NSt	45	11.6 (10.2-13.1)	NSt
Hassan, 2004	umol/l	>2m	172	14.5 (13.78-15.35) ♂	172	12.0 (11.42-12.64) ♂	p<0.0005	--	--	--	--	--	--
Parnetti, 2004	umol/l	NSt	50	13.9 ±5.4	152	8.1 ±2.5	p<0.0001	43	17.8 ±13.5	p=0.046	31	13.0 ±2.5	≠
Khan, 2007	umol/l	>3m	47	15.14 ±5.59	38	12.49 ±4.15	p=0.029	--	--	--	--	--	--
Khan, 2008	umol/l	On	152	16.2 ±11.6	179	11.8 ±5.7	p<0.001	40	11.6 ±4.8	p<0.005	72	14.3 ±6.3	≠
Yokokawa, 2008	nmol/ml	On	55	10.6 {6.0-50.0}	--	--	--	87	11.2 {5.0-26.3}	≠	19	9.4 {5.7-26.4}	≠
Beer, 2011	umol/l	~63h	25	10.1 ±3.6	--	--	--	19	9.9 ±4.9	≠	43	10.2 ±3.9	≠
Jeong, 2011	umol/l	3d	83	11.3 ±3.8	135	11.2 ±3.4	p=0.871	--	--	--	--	--	--
Pavlovic, 2011	umol/l	1-6m	95	14.4 ±5.0	41	8.9 ±3.9	p<0.0001	--	--	--	--	--	--
von Willebrand factor													
Bath, 1998	IU/dl	<48h	40	144 [19]	25	114 [16]	NSt	28	140 [15] ♣	≠1	39	158 [25] ♥	≠1
Kozuka, 2002	%	<48h	27	165 (123-206)	86	132.5 (102-166)	p=0.011	16	176.5 (145-195.5)	NSt	9	223 (166-253)	NSt
		1m	27	176 (143.5-202.3)	--	Used <48h data	p<0.001	16	190.5 (167.5-251.5)	NSt	5	182.5 (172-228)	NSt
Licata, 2009	ng/ml	36h	46	6 (3-8)	123	4 (3-8)	§	50	8 (6-10)	p=0.005^	20	10 (5-12)	p=0.005^
Tuttolomondo, 2009a	ng/ml	<12h	32	5 (2-9)	102	4 (3-9)	§	41	7 (4-11)	p=0.005^	29	12 (6-13)	p=0.005^
Beer, 2011	%	~63h	25	164 ±56.7	--	--	--	19	163.0 ±67.3	≠	43	158.8 ±61.0	≠
Hanson, 2011	IU/dl	<10d	123	213 (197-229) ♂	599	180 (145-245) ♠	p<0.01	69	253 (230-279) ♂	Sig0	94	263 (242-286) ♂	Sig0
		3m	123	201 (189-215) ♂	--	Used <10d data	≠	69	NSt	NSt	94	240 (224-256) ♂	Sig0
Fibrinogen													
Kilpatrick, 1993	g/l	<48h	9	3.9 ±1.1	♦	2.0-5.0	≠	10	4.1 ±1.2	NSt	--	--	--
Beamer, 1995	mg/dl	4d	23	377 ±133	20	334 ±39	§	12	452 ±91	≠	6	493 ±184	≠
Bath, 1998	g/l	<48h	56	4.65 [0.7]	33	3.7 [0.7]	NSt	38	4.6 [0.75] ♣	≠1	53	4.8 [0.9] ♥	≠1
Kataoka, 2000	mg/ml	<48h	58	316 ♠	32	315 ±16.2	NSt	41	415 ♠	p<0.0001	38	340 ♠	NSt
		1w	58	400 ♠	--	--	--	41	520 ♠	p<0.0001	38	490 ♠	p<0.001
		3w	58	350 ♠	--	--	--	41	480 ♠	p<0.0001	38	450 ♠	NSt
Salobir, 2002	g/l	chron	16	2.6 ±0.4	47	2.3 ±0.4	p<0.05	--	--	--	--	--	--
Jood, 2008	g/l	<10d	123	3.55 (2.91-4.18)	599	2.91 (2.61-3.30)	p<0.001	69	4.14 (3.31-5.10)	NSt	94	3.86 (3.35-4.61)	NSt
		3m	123	3.27 (2.89-3.69)	--	Used <10d data	p<0.001	69	3.64 (3.06-4.65)	NSt	94	3.43 (2.92-4.09)	NSt
Alvarez-Perez, 2011	mg/dl	<48h	50	322.5 (270-390)	50	281 (255-319.5)	NSt	50	325 (287-374)	≠	50	340 (293-408.5)	≠
Beer, 2011	g/l	~63h	25	4.1 ±0.8	--	--	--	19	4.3 ±1.1	≠	43	4.2 ±0.9	≠
Zhang, 2011	g/l	On	262	2.96 ±1.04	--	--	--	364*	3.23 ±1.37	p=0.02	--	--	--
CRP													
Ladenvall, 2006	mg/l	<10d	124	3.08 (1.52-5.79)	600	1.61 (0.85-3.38)	p<0.001	73	4.66 (1.79-13.9)	NSt	98	7.07 (2.39-17.8)	NSt
		3m	124	2.65 (1.26-4.82)	--	Used <10d data	p<0.001	73	3.48 (1.42-9.99)	NSt	98	2.66 (1.07-5.46)	NSt
Montaner, 2008	ug/ml	8h	128	9.8 (4.6-23.7)	--	--	--	151	8.7 (4.1-21.7)	≠	259	11.7 (4.6-28.6)	≠
Nakase, 2008	ng/dl	On	42	103.4 ±138.4	--	--	--	20	140.6 ±133.3	NSt	30	174.2 ±262.9	NSt

Yokokawa, 2008	mg/l	On	55	0.095 {0.029-0.95}	--	--	--	87	0.092 {0.029-8.97}	≠	19	0.115 {0.029-1.43}	≠
Licata, 2009	mg/dl	36h	46	3.1 (1.4-4.1)	[123]	1.3 (0.8-1.5)	§	50	3.2 (1.3-4.0)	p=0.35^	20	3.4 (1.5-4.3)	p=0.35^
Alvarez-Perez, 2011	mg/dl	<48h	50	0.32 (0.16-0.68)	50	0.18 (0.08-0.30)	NSt	50	0.58 (0.24-1.09)	p=0.01	50	0.36 (0.24-0.97)	NSt
Beer, 2011	mg/l	~63h	25	4.6 ±4.7	--	--	--	19	11.4 ±25.4	≠	43	14.1 ±19.7	≠
Turgut, 2011	mg/l	1w	20	1.59 ±1.63	37	0.19 ±0.24	p<0.0001	31	3.34 ±4.58	≠	21	2.93 ±4.56	≠
ICAM													
Castellanos, 2002	pg/ml	~10h	[113]	187 (172-223)	43	167 (140-207)	p=0.015	--	--	--	--	--	--
Hassan, 2003	ng/ml	210d	[110]	425.3 (395-457) ♂	50	341.9 (320-364) ♂	p<0.0005	--	--	--	--	--	--
Tuttolomondo, 2009a	ng/ml	<12h	32	20.51 (15-23)	[102]	10.9 (12-16.1)	§	41	23.8 (15.9-24)	p=0.57^	29	22.52 (18.55-23)	p<0.57^
Tuttolomondo, 2009b	ng/ml	<72h	46	19.91 (15-23)	[123]	15.9 (12-18.1)	§	50	20.8 (15.9-24)	p=0.56^	20	19.52 (18.55-23)	p=0.56^
Supanc, 2011	ng/ml	<24h	43	358 {14.8-673}	93	385.7 {213.7-464}	NSt	67	376.5 {40-745}	NSt	--	--	--
VCAM													
Tuttolomondo, 2009a	ng/ml	<12h	32	16 (15-20)	[102]	10 (7-15)	§	41	21 (13-22)	p=0.52^	29	20 (14.7-24)	p<0.52^
Tuttolomondo, 2009b	ng/ml	<72h	46	17 (15-20)	[123]	14 (13-17)	§	50	20 (15-24)	p=0.53^	20	20 (15.7-26)	p=0.53^
Supanc, 2011	ng/ml	<24h	43	675 (445-1210)	93	688 (555-850)	NSt	67	730 (90-1810)	NSt	--	--	--
E-selectin													
Kozuka, 2002	ng/ml	<48h	27	53.9 (34.8-65.5)	86	38.4 (28.6-46)	p=0.001	16	44.3 (30.3-69)	NSt	9	39.7 (31.8-54.6)	NSt
		1m	27	44.2 (34.9-55.8)	--	Used <48h data	p=0.09	16	55.5 (30-68.7)	NSt	5	41.7 (29.2-57.2)	NSt
Tuttolomondo, 2009a	ng/ml	<12h	32	3 (2-5)	[102]	2 (1-2)	§	41	4 (2-6)	p<0.68^	29	2.25 (1-4.5)	p<0.68^
Tuttolomondo, 2009b	ng/ml	<72h	46	2 (2-4)	[123]	2 (1-2)	§	50	3 (2-4)	p=0.80^	20	2.75 (2-3.5)	p=0.80^
Beer, 2011	ng/ml	~63h	25	31.5 ±19.7	--	--	--	19	22.4 ±17.0	≠	43	25.4 ±17.6	≠
P-selectin													
Bath, 1998	ng/ml	<48h	40	300 [108]	26	324 [121]	NSt	28	265 [101] ♣	≠1	39	408 [101] ♥	p<0.05
Kozuka, 2002	ng/ml	<48h	27	41.2 (33.9-62.5)	86	24.6 (20.8-30.5)	p<0.001	16	48.9 (39.5-62.8)	NSt	9	70.2 (35.7-83.0)	NSt
		1m	27	39.7 (29.3-60.0)	--	Used <48h data	p<0.001	16	65.1 (40.2-76.8)	NSt	5	64.1 (45.9-87.8)	NSt
Tsai, 2009	%	<48h	32	4.9 (1.7-8.2)	--	--	--	22	9.9 (4.3-12.7)	p<0.001	--	--	--
		7d	32	4.4 (1.7-5.5)	--	--	--	22	8.6 (3.0-11.5)	p<0.05	--	--	--
		30d	32	3.8 (1.1-6.0)	--	--	--	22	7.7 (4.0-11.3)	p<0.05	--	--	--
		90d	32	2.3 (0.9-3.4)	--	--	--	22	5.1 (1.9-7.1)	≠	--	--	--
Tuttolomondo, 2009a	ng/ml	<12h	32	4 (2.2-7)	[102]	3.1 (2.1-4)	§	41	4.05 (2-6)	p<0.34^	29	3.1 (1.3-6.3)	p<0.68^
Tuttolomondo, 2009b	ng/ml	<72h	46	4 (2.4-6)	[123]	3.1 (2.1-4)	§	50	4.95 (2-6.5)	p=0.49^	20	3.4 (1.46-6.43)	p=0.49^
Ilhan, 2010	ng/ml	0-5d	30	44.03 ±18.7	30	48.06 ±9.19	p=0.243	--	--	--	--	--	--
		15d	30	42.70 ±23.80	--	--	--	--	--	--	--	--	--
		30d	30	42.86 ±16.05	--	--	--	--	--	--	--	--	--
Turgut, 2011	%	1w	20	7.26 ±2.49	37	3.89 ±4.16	p=0.02	31	7.49 ±5.06	≠	21	7.16 ±3.56	≠
TNF-α													
Castellanos, 2002	pg/ml	~10h	[113]	8.2 (6.4-15.3)	43	7.0 (5.7-8.4)	p=0.001	--	--	--	--	--	--
Domac, 2007	pg/ml	12h	19	41.7±26.3$	22	16.7 ±5.5	p<0.0001	16	39.2 ±25.3$$	≠	--	--	--
Licata, 2009	pg/ml	36h	46	19.4 (9-23)	[123]	3.7 (1.1-4.3)	§	50	27.5 (13-40.5)	p<0.0001	20	38.5 (22.2-46)	p<0.0001
Tuttolomondo, 2009a	pg/ml	<12h	32	18.4 (11-23)	[102]	5.1 (1.1-4.3)	§	41	29.5 (15-44.5)	p<0.0001	29	37.2 (21.2-48)	p<0.0001
Nakase, 2008	pg/ml	On	42	1.9 ±1.7	--	--	--	20	1.5 ±0.6	≠	30	1.7 ±1.5	≠
IL-6													
Beamer, 1995	pg/ml	4d	23	2.8 ±2.8	20	1.0 ±0.9	§	12	5.4 ±2.7	≠	6	9.9 ±6.6	p<0.01
Vila, 2000	pg/ml	<24h	33	9.6 ±9.3	--	--	--	84	24.5 ±14.2	p<0.01	76	18.0 ±16.4	p<0.01
Castellanos, 2002	pg/ml	~10h	[113]	13.9 (9.2-23.8)	43	3.1 (1.3-4.1)	p<0.001	--	--	--	--	--	--
Salobir, 2004	pg/ml	3.5y	16	1.6 (0-2.6)	47	1.4 (0-2.0)	≠	--	--	--	--	--	--
Guldiken, 2008	pg/ml	<72h	16	8.79 ±3.73	23	4.68 ±3.0	p<0.01	12	4.28 ±3.97	p<0.01	--	--	--
Domac, 2007	pg/ml	12h	19	43.8±18.5$	22	15.1 ±4.9	p<0.0001	16	34.1 ±17.05$$	≠	--	--	--
Nakase, 2008	pg/ml	On	42	3.0 ±3.7	--	--	--	20	3.3 ±3.1	≠	30	3.6 ±2.6	≠
Licata, 2009	pg/ml	36h	46	4.0 (2.0-9.0)	[123]	9 (2.90-18)	§	50	8 (4-12)	p=0.003^	20	11 (5.5-19)	p=0.003^
Tuttolomondo, 2009a	pg/ml	<12h	32	5 (2-8)	[102]	8 (3.1-12)	§	41	7 (4-11)	p=0.003^	29	12 (6.5-18)	p=0.003^

Values in units given, either as means alone **or** means ±SD **or** medians (IQR) **or** medians (Q1-Q3) **or** medians [semi-quartiles] **or** medians {min-max} **or** ♂ geometric mean with 95% CI.

-- = not investigated / measured. NSt = not stated

AT = atherothrombotic stroke, CE = cardioembolic stroke

p1 = lacunar versus non-stroke controls, p2 = lacunar versus atherothrombotic stroke, p3 = lacunar versus cardioembolic stroke

≠ no p value given but authors stated "not statistically significant" (typically against p<0.05)

≠1 not significant across multiple stroke subtypes but no specific pairwise analysis to lacunar stroke, assumed not sig. ≠2 not significant versus the non-stroke control 1d value

Time is average time to blood draw following initial stroke event, either mean or median. On = blood markers measured on admission to hospital. Chron = authors state "a chronic, stable phase after LACI".

* Non-lacunar strokes (ie, includes some cardioembolic strokes)

♦ Used a laboratory normal range to compare lacunar to.

♠ Authors only present results graphically; this systematic review has read data off the graph.

♣ PACI (not AT) ♥ TACI (not CE)

$ small sub-cortical infarct, $$ small cortical infarct

Sig0 Stated as significant but no p value given

Sig1 Lacunar to non-stroke control not stated although all patients (TACI, PACI, LACI) differed from non-stroke controls, p=0.0001

Sig2 Lacunar to non-stroke control not stated although all patients (TACI, PACI, LACI) differed from non-stroke controls, p<0.01

& p value compares non-CE strokes (lacunar and AT) to CE

§ All strokes (lacunar and non-lacunar) are compared to non-stroke controls, and so cannot isolate a p value comparison for lacunar to healthy control

‡ Lacunar versus non-lacunar (AT+CE+UDE)

^ Analysis of variance across multiple stroke groups (LAC, AT, CE) rather than solely lacunar to other individual subtype.

Discussion

This review assessed blood markers of coagulation, fibrinolysis, endothelial dysfunction and inflammation in lacunar stroke versus non-stroke controls and other ischaemic stroke subtypes. While many markers were higher in lacunar stroke than in non-stroke controls, they were mostly lower in lacunar versus non-lacunar stroke. A brief summary follows.

Coagulation/fibrinolysis

t-PA/PAI were significantly higher in lacunar stroke versus non-stroke, acutely and chronically and did not differ between lacunar and non-lacunar stroke, acutely or chronically.

Fibrinogen did not differ between lacunar stroke and non-stroke acutely although we only used a single time point from Kataoka et al.[98] (bloods drawn at <24). If we consider their second sample (at 7 days) as a further acute measurement, lacunar stroke tends towards being significantly higher than non-stroke. Fibrinogen was significantly higher in lacunar stroke versus non-stroke chronically. Fibrinogen was significantly lower in lacunar versus non-lacunar stroke, acutely and chronically. However, studies excluded from the meta-analysis tended to show no overall difference between lacunar and non-lacunar stroke and chronic data came from a single study.

D-dimer was significantly higher in lacunar stroke versus non-stroke, acutely and chronically, and significantly lower in lacunar versus non-lacunar stroke, acutely and chronically.

Endothelial dysfunction

Homocysteine was significantly higher in lacunar stroke versus non-stroke, acutely and chronically, did not differ between lacunar and non-lacunar stroke acutely (but no chronic phase studies).

vWF was significantly higher in lacunar stroke versus non-stroke, acutely, with conflicting evidence chronically. vWF was significantly lower in lacunar than non-lacunar stroke acutely (2 studies) with conflicting but non-meta-analyzable evidence in other studies both acutely and chronically.

E-selectin was significantly higher in lacunar stroke versus non-stroke acutely (only one study) but not chronically and did not differ between lacunar and non-lacunar stroke, either acutely or chronically (only one study).

P-selectin was significantly higher in lacunar stroke versus non-stroke acutely in some but not all studies, and in the only study that reported a chronic measurement. P-selectin did not differ between lacunar and non-lacunar stroke, either acutely or chronically (only one study).

ICAM was significantly higher in lacunar stroke versus non-stroke, acutely and chronically (only one study), did not differ between lacunar and non-lacunar stroke acutely, with no studies chronically.

VCAM did not differ between lacunar stroke and non-stroke, nor between lacunar and non-lacunar stroke acutely. There were no studies chronically.

Inflammation

CRP was significantly higher in lacunar stroke versus non-stroke, acutely and chronically (only one study) and did not differ between lacunar and non-lacunar stroke acutely or chronically (only one study).

TNF-α was significantly higher in lacunar stroke versus non-stroke acutely with no studies chronically. There was conflicting evidence on levels of TNF-α in lacunar versus non-lacunar stroke acutely with no studies chronically.

IL-6 was significantly higher in lacunar stroke versus non-stroke acutely but did not differ chronically. IL-6 was significantly lower in lacunar versus non-lacunar stroke acutely, but there were no chronic phase studies.

This suggests that plasma marker elevation in lacunar stroke is likely to reflect the process of having a stroke rather than that systemic inflammation or endothelial dysfunction is specific to lacunar stroke. The available data were limited and do not exclude the possibility that peripheral inflammatory or endothelial dysfunction processes are associated with lacunar stroke specifically.

There were limitations in the studies. Most were small, with varying methods and an inconsistent definition of 'lacunar stroke', as highlighted previously[121]. Papers reviewed used the term lacunar stroke to reflect a clinical entity, ie clinical presentation with a stroke, but definitions varied. We were not able to differentiate different

mechanisms of lacunar stroke. Most lacunar strokes are due to recent small subcortical infarcts (RSSI), and most RSSI relate to intrinsic small vessel disease. However, RSSI also arise from atherothromboembolism (large artery) or cardioembolism in a small proportion of patients and it was not possible to differentiate these cases.

There was heterogeneity across several aspects of the methods. Many used TOAST[85] but as this uses risk-factors to categorize patients it potentially introduces classification bias. A patient with an unclear diagnosis of lacunar stroke but concurrent hypertension or diabetes might (rightly or wrongly) be classified as 'lacunar' using this system although hypertension and diabetes were equally prevalent risk factors between ischaemic stroke subtypes in 21,980 stroke patients when subtypes were classified without risk factors[20]. Several did not report on whether their findings achieved statistical significance; in the absence of an explicit statement, we report this as *not stated*. Some studies drew blood after overnight fasting, others collected non-fasting blood. Studies used different units of measurement and assay methods. Timing of blood draw in relation to stroke varied but is important to account for each marker's individual 'response curve' which changes over time. Fassbender and colleagues[122] found levels of IL-6 to rise rapidly following onset of ischaemic stroke, reaching a plateau at 10 hours until 3 days before returning to normal by day 7. Between-study heterogeneity on time to blood draw complicates subsequent analysis although meta-analyses use within-study data thus minimising any effect of between-study variation.

Our review had limitations. We did not study markers in cerebrospinal fluid. We did not review the association of marker levels with lesion size or clinical outcome as data were sparse. Ahmad et al.[123] found markers of neuronal damage correlated with infarct size which might explain why marker levels in non-lacunar stroke were frequently

higher than in lacunar stroke in the acute phase. We were not able to analyse differences between groups reported as top versus bottom quantiles. There are also potential sources of bias in our review: we did not review non-English language studies, the studies meta-analysed were observational and also we did not use funnel plots to assess for publication bias. We did not formally test the methodological quality of studies reviewed by sensitivity analysis to investigate impact on effect size.

Our review had strengths including assessment of differences between stroke subtypes, quality assessment of included studies (but no formal testing for impact on effect size), meticulous extraction of data and meta-analysis thereon, wherever suitable data were available. Previous reviews compared lacunar stroke to non-stroke controls only and therefore did not distinguish lacunar stroke specifically from stroke in general.

To determine if there is a difference in coagulation, fibrinolysis, endothelial dysfunction and inflammation in lacunar versus other stroke subtypes requires a large prospective study of blood markers in accurately phenotyped patients with lacunar versus non-lacunar stroke classified using non-risk factor based definitions. Future studies should clearly define and diagnose lacunar stroke, avoid subtyping stroke using risk factor based classifications, and obtain blood several weeks post stroke to avoid confounding from an acute phase response.

Chapter 3: Plasma biomarkers of inflammation, endothelial function and haemostasis in cerebral small vessel disease: the Mild Stroke Study

[now published: Wiseman et al. *Cerebrovasc Dis.* 2015;40:157–64]

Introduction

Plasma biomarkers of inflammation, endothelial dysfunction and haemostasis may provide mechanistic insights to the cause of lacunar ischaemic stroke via implicated processes such as systemic inflammation, blood-brain barrier failure or occlusive microthrombus[18,67–70]. Plasma biomarkers may predict outcome after stroke[124] and could have a role in management of stroke patients[125].

In chapter 2, a systematic review and meta-analysis showed most plasma biomarkers were raised in lacunar stroke versus non-stroke controls, probably reflecting the acute response to having a stroke. However, when lacunar stroke was compared to other ischaemic stroke subtypes to control for having a stroke, some biomarkers had lower values (D-dimer, vWF, fibrinogen and IL-6) but many other biomarkers either showed no difference to the other stroke subtypes, or data were limited or inconclusive.

Magnetic resonance (MR) imaging features of SVD include white matter hyperintensities (WMH) and are associated with ischaemic and haemorrhagic stroke and dementia[21]. WMH predict an increased risk of stroke[21] and are associated with poor functional outcomes following stroke[126].

The relationship between plasma biomarkers and imaging biomarkers of SVD is not fully understood. Systematic reviews[68,74,127] are impeded by between-study heterogeneity in the source studies, and differ in their findings. Generally, plasma

biomarkers are raised in lacunar stroke versus non-stroke healthy controls (used in most studies) but differences here are unsurprising, especially in the acute phase of stroke. The situation is less clear when lacunar stroke is compared to other ischaemic stroke subtypes.

In two large population studies of subjects without stroke, higher inflammatory biomarkers were independently associated with higher WMH volumes[67,128] but not in three other studies[129–131]. Biomarkers of endothelial activation were associated with WMH in cross sectional analysis[132] and with WMH progression[69]. Flow-mediated dilatation studies have shown endothelial dysfunction in lacunar stroke versus non-stroke controls[133].

Prior stroke studies often take the plasma samples too early making it difficult to isolate underlying trends independent from an acute phase response. Few studies assessed a range of biomarkers simultaneously in one population.

The purpose of this study was (1) to determine if there were differences in levels of plasma biomarkers of a) inflammation, b) endothelial dysfunction or c) haemostasis between lacunar and cortical stroke subtypes, well after the acute event, as representative of three potential SVD mechanisms, adjusted for age and major vascular risk factors; (2) to update our meta-analysis and place current findings into context; and (3) to assess the association between the three plasma biomarker groups and WMH, irrespective of stroke subtype.

Methods

Our definition of SVD is in accordance with *Standards for reporting vascular changes on neuroimaging* (STRIVE) neuroimaging reporting guidelines[19].

Patients

We prospectively recruited patients as consecutively as possible who presented with ischaemic stroke of lacunar or mild (ie, non-disabling) cortical subtype seen at a regional stroke service, as detailed previously[134]. Patients with cortical stroke acted as controls because they have many similar risk factors, medications and extent of damage due to the stroke to patients with lacunar stroke, thus controlling for potential confounders and allowing us to differentiate findings specific to SVD. We excluded patients with contraindications to MR, haemorrhagic stroke or severe stroke, ie, disabling total anterior circulation stroke. The study was approved by the local research ethics committee (2002/8/64) and all patients gave written informed consent.

Patient investigations

Patients were clinically assessed at presentation and underwent MR brain imaging at 1.5T, carotid Doppler ultrasound and electrocardiogram. We recorded past medical histories including hypertension, diabetes, hypercholesterolemia and smoking, and measured blood pressure and blood lipids as per the usual stroke patient assessment.

Stroke subtype

We assessed stroke severity with the National Institute for Health Stroke Scale (NIHSS)[135] (but did not use NIHSS as selection criteria) and classified the stroke

clinical syndrome (lacunar or cortical) according to the Oxfordshire Community Stroke Project (OCSP)[15]. We defined "lacunar stroke" as per the classical clinical lacunar syndromes (pure motor weakness or sensory loss or both in face and arm, arm and leg or all three, ataxic hemiparesis or clumsy hand dysarthria syndrome). We defined "mild cortical stroke" as a maximum clinical deficit of either: weakness or sensory loss in the face, arm or leg, or loss of higher cerebral function (dysphasia or neglect), or weakness in more than one limb in the presence of loss of higher cerebral function (all in keeping with a partial anterior circulation stroke), or a homonymous hemianopia suggestive of occipital cortical infarct (in keeping with a cortical posterior circulation stroke).

We then assessed whether a recent infarct on MR was firstly present and secondly whether it was cortical or lacunar. We based the final stroke subtype classification on both the clinical and radiological classification. Where the clinical classification differed from the radiological classification, the radiological classification was used – using clinical criteria alone can result in misclassification of infarcts in up to 20% of cases[136]. Where an infarct on imaging was absent, an expert panel with all available information assigned the final stroke subtype.

Plasma biomarkers

All patients had blood sampled after a minimum of one and maximum of three months following stroke to avoid the acute phase. Samples were spun and frozen for batch analysis, blind to clinical data. We measured markers of inflammation (C-reactive protein (CRP), tumour necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6)), endothelial activation (von Willebrand factor (vWF) and intracellular adhesion

molecule-1 (ICAM)) and thrombotic/fibrinolytic activity (fibrinogen, tissue plasminogen activator antigen (t-PA) and D-dimer). Intra- and inter-assay variation on biomarker testing was between 3.3% and 12.5% (Table 3.1).

Table 3.1 Intra- and inter-assay variation on biomarker testing

Blood marker	Method	Units	Intra-assay CV	Inter-assay CV
Inflammation				
CRP	Immunonephelometry (Prospec, Dade Behring Milton Keynes, UK)	mg/l	4.7%	8.3%
TNF-α	ELISA (R&D Systems, Abingdon, UK)	pg/ml	8.4%	12.5%
IL-6	ELISA (R&D Systems, Abingdon, UK)	pg/ml	7.5%	8.9%
Endothelial dysfunction				
ICAM	ELISA (R&D Systems, Abingdon, UK)	ng/ml	3.6%	7.4%
vWF	ELISA (DAKO, High Wycombe, UK)	IU/dl	3.3%	4.2%
Thrombosis/fibrinolysis				
Fibrinogen	Immunonephelometry (Prospec, Dade Behring Milton Keynes, UK)	g/l	7.5%	8.9%
t-PA	ELISA (Biopool AB, Umea Sweden)	ng/ml	6.6%	6.5%
D-dimer	ELISA (Biopool AB, Umea Sweden)	ng/ml	4.7%	5.2%

CRP = C-reactive protein, CV = coefficients of variation, ELISA = enzyme-linked immunosorbent assay, ICAM = intracellular adhesion molecule-1, IL-6 = interleukin-6, TNF-α = tumour necrosis factor alpha, t-PA = tissue plasminogen activator, vWF = von Willebrand factor.

Image analysis

All scans were reviewed by a neuroradiologist for the index infarct and rated for SVD features using standardized scales[137–139] including the Fazekas scale for WMH. A quantitative, volumetric measure of WMH (in mm^3) was also calculated, as described previously[140]. We corrected for head size by dividing the quantitative WMH load by the intracranial volume. Visually rated WMH correlated strongly with quantitative WMH (r=0.84, 95% CI 0.77 to 0.89).

Statistical analysis

We assessed differences in patient demographics and plasma biomarkers between stroke subtypes using Student's *t* test, Mann-Whitney U test and Chi-squared test (χ^2), as appropriate. Quantitative WMH and some plasma biomarkers (CRP, TNF-α, IL-6, D-dimer) were not normally distributed so we log transformed these data.

We explored the association between stroke subtypes and plasma biomarkers with multiple linear regression to control for age, sex and vascular risk factors.

We used multiple linear regression to assess the contribution of plasma biomarkers in explaining variance in quantitative WMH volume (n=98; 27 scans were unavailable for WMH quantification), irrespective of stroke subtype. We repeated the modelling with visually rated WMH, for which the full dataset was available (n=125).

We used standardized units (mean=0, sd=1) for all plasma biomarkers in the regression models. The standardized data have no units and are on the same scale, so different biomarkers can be added together into summed variables which reduces the number of predictor variables to help avoid model over-fitting. We verified the correlation between the components of the summed variables. The summed variables were: Inflammation (INF) (CRP + TNF-α + IL-6), Endothelial dysfunction (END) (vWF + ICAM) and Thrombosis (THR) (t-PA + D-dimer + fibrinogen).

We fitted a baseline model with quantitative WMH volume as the outcome measure and age, sex, hypertension and smoking status as the predictor variables. Patients with a past history of tobacco use were classified as non-smokers if they were non-smokers at time of stroke.

We fitted four further models:

(Model 1) baseline + inflammation (INF)

(Model 2) baseline + endothelial dysfunction (END)

(Model 3) baseline + thrombosis/fibrinolysis (THR)

(Model 4) baseline + INF + END + THR

We compared each model for improvement over baseline. Model improvement was defined as a reduction in residual standard error (RSE) and increase in adjusted r-squared (R^2).

We checked for multicolinearity between predictor variables using variance inflation factor. We checked model assumptions as follows: independence, linearity, constancy of variance and normality in the residuals. A p value of <0.05 was considered significant. All analyses were performed with the statistical programming language R version 3.0.1 (http://www.r-project.org/)[141].

Meta-analysis

We used the Review Manager 5 software (The Cochrane Collaboration) to update our prior meta-analysis (Chapter 2)[127], calculating the standardized mean difference using the inverse variance method and a fixed effects model with 95% CI.

Results

We recruited 125 patients, 65 with lacunar stroke and 60 with cortical stroke. The mean age of the total cohort was 66.4 ± 11.4 years and the median NIHSS was one (Q1-Q3 1-2). The median time from stroke onset to blood sampling was 54.4 (Q1-Q3 36-74) days. Patient characteristics and plasma biomarkers by stroke subtype are listed in Table 3.2. The lacunar group had fewer men (39 *v* 51, p=0.004), were younger (64 *v* 69 years, p=0.015) and suffered less atrial fibrillation (2 *v* 9, p=0.042) compared with the cortical group (Table 3.2).

Table 3.2 Comparing patient characteristics and plasma biomarkers between lacunar and cortical stroke

	Lacunar stroke (n=65)	**Cortical stroke (n=60)**	**p value**
Male, n (%)	39 (60%)	51 (85%)	0.004 *
Age, mean (SD) years	64.1 (11.4)	69.0 (10.9)	0.015 *
Hypertension, n (%)	37 (57%)	39 (65%)	0.458
Diabetes, n (%)	14 (21.5%)	5 (8.3%)	0.071
Current smoker, n (%)	24/64 (37.5%)	13/59 (22.0%)	0.095
NIHSS, median (Q1 – Q3)	2 (1 – 3)	1 (0.75 – 2)	0.404
Time to sample, median (Q1 – Q3) days	56 (38 – 74)	52 (36 – 77)	0.894
Ischaemic heart disease, n (%)	8 (12.3%)	16 (26.6%)	0.070
Atrial fibrillation, n (%)	2 (3.1%)	9 (15%)	0.042 *
Hyperlipidemia, n (%)	26/64 (40.6%)	23 (38.3%)	0.939
Total cholesterol, mean (sd) mmol/l	5.07 (1.10) (n=57)	5.06 (1.13) (n=53)	0.949
Positive family history of stroke, n (%)	10/64 (15.6%)	4/58 (6.9%)	0.220
Inflammation			
CRP, median (Q1 – Q3) mg/L	1.37 (0.84 – 3.44)	1.73 (0.97 – 3.54)	0.748
TNF-α, median (Q1 – Q3) pg/mL	0.92 (0.72 – 1.33)	0.88 (0.76 – 1.23)	0.972
IL-6, median (Q1 – Q3) pg/mL	2.57 (1.91 – 4.12) (n=64)	2.58 (1.90 – 3.77)	0.994
Endothelial dysfunction			
ICAM, mean (SD) ng/mL	162.78 (57.97)	159.27 (46.1) (n=56)	0.711
vWF, mean (SD) iu/dL	129.31 (41.49)	131.7 (39.2)	0.741
Thrombosis/fibrinolysis			
Fibrinogen, mean (SD) g/L	3.84 (0.61) (n=64)	3.93 (0.67) (n=59)	0.452
t-PA, mean (SD) ng/mL	7.39 (3.13)	8.59 (2.92)	0.029 *
D-dimer, median (Q1 – Q3) ng/mL	100 (73 – 157)	128.5 (73.25 – 182.5)	0.498

* $p<0.05$.

CRP = C-reactive protein, ICAM = intracellular adhesion molecule-1, IL-6 = interleukin-6, NIHSS = National Institute Health Stroke Scale, TNF-α = tumour necrosis factor alpha, t-PA = tissue plasminogen activator, vWF = von Willebrand factor.

Plasma biomarker association with lacunar stroke

The lacunar group had lower t-PA levels compared with the cortical group (7.39 *v* 8.59 ng/mL, p=0.029) in unadjusted analyses (Table 3.2) and after adjustment for age, sex, hypertension, smoking, diabetes and atrial fibrillation (p=0.035, Table 3.3). There were no differences in the other plasma biomarkers between lacunar stroke and cortical stroke whether adjusted or not (Tables 3.4–3.6).

Table 3.3 Association of tissue plasminogen activator (t-PA) with lacunar stroke subtype (n=125)

	Regression coefficient (95% CI)	**p value**
Lacunar stroke subtype	-1.312 (-2.531 to -0.093)	0.035 *
Age	-0.017 (-0.073 to 0.038)	0.530
Male sex	0.542 (-0.740 to 1.824)	0.404
Hypertension	0.393 (-0.806 to 1.593)	0.517
Smoking	1.027 (-0.277 to 2.333)	0.121
Diabetes	0.266 (-1.324 to 1.855)	0.741
Atrial fibrillation	0.240 (-1.854 to 2.334)	0.820
* p<0.05.		

Table 3.4 Association of plasma biomarkers of inflammation (CRP, TNF-α and IL-6) with lacunar stroke subtype (n=125)

	Regression coefficient (95% CI)	**p value**
CRP		
Lacunar stroke subtype	-1.441 (-4.711 to 1.830)	0.385
Age	-0.024 (-0.173 to 0.126)	0.753
Male sex	1.000 (-2.533 to 4.533)	0.576
Hypertension	1.004 (-2.390 to 4.399)	0.559
Smoking	-0.182 (-3.760 to 3.396)	0.920
TNF-α		
Lacunar stroke subtype	0.288 (-0.203 to 0.779)	0.247
Age	-0.008 (-0.030 to 0.014)	0.473
Male sex	0.004 (-0.526 to 0.534)	0.988
Hypertension	-0.162 (-0.671 to 0.347)	0.530
Smoking	-0.445 (-0.982 to 0.092)	0.103
IL-6		
Lacunar stroke subtype	0.366 (-0.423 to 1.155)	0.360
Age	0.029 (-0.007 to 0.065)	0.114
Male sex	0.365 (-0.486 to 1.217)	0.397
Hypertension	-0.068 (-0.891 to 0.754)	0.869
Smoking	0.425 (-0.439 to 1.290)	0.332
CRP = C-reactive protein, IL-6 = interleukin-6, TNF-α = tumour necrosis factor alpha.		

Table 3.5 Association of plasma biomarkers of endothelial dysfunction (ICAM and vWF) with lacunar stroke subtype (n=125)

	Regression coefficient (95% CI)	**p value**
ICAM		
Lacunar stroke subtype	2.552 (-18.420 to 23.524)	0.810
Age	-0.193 (-1.144 to 0.757)	0.688
Male sex	7.954 (-14.608 to 30.517)	0.486
Hypertension	-3.688 (-25.350 to 17.974)	0.737
Smoking	11.386 (-11.437 to 34.210)	0.325
vWF		
Lacunar stroke subtype	5.935 (-9.076 to 20.946)	0.435
Age	1.225 (0.537 to 1.912)	0.000 ***
Male sex	5.275 (-10.937 to 21.488)	0.520
Hypertension	-3.808 (-19.386 to 11.770)	0.629
Smoking	1.331 (-15.089 to 17.752)	0.873
*** p<0.001. ICAM = intracellular adhesion molecule-1, vWF = von Willebrand factor.		

Table 3.6 Association of plasma biomarkers of thrombosis (fibrinogen and D-dimer) with lacunar stroke subtype (n=125)

	Regression coefficient (95% CI)	**p value**
Fibrinogen		
Lacunar stroke subtype	-0.108 (-0.351 to 0.135)	0.382
Age	0.006 (-0.004 to 0.017)	0.238
Male sex	-0.000 (-0.262 to 0.262)	0.998
Hypertension	0.063 (-0.189 to 0.317)	0.620
Smoking	0.440 (0.174 to 0.706)	0.001 ***
D-dimer		
Lacunar stroke subtype	-22.986 (-90.559 to 44.587)	0.502
Age	2.094 (-1.000 to 5.188)	0.183
Male sex	12.475 (-60.505 to 85.456)	0.736
Hypertension	5.056 (-65.070 to 75.182)	0.887
Smoking	56.899 (-17.019 to 130.817)	0.130
*** p<0.001.		

To determine if the reduced t-PA was related to smoking, we repeated the analysis in non-smokers only (lacunar stroke, n=40 versus cortical stroke, n=46). t-PA levels remained lower in lacunar stroke (Table 3.7). The difference became nonsignificant when adjusted for age, sex, hypertension and diabetes, but the change was slight, the regression coefficients and 95% confidence intervals were similar[142] being -1.31 (95% CI -2.53 to -0.09) versus -1.37 (-2.84 to 0.09) and may reflect the reduced sample size.

Table 3.7 Comparing patient characteristics and plasma biomarkers between lacunar and cortical stroke in non-smokers

	Lacunar stroke (n=40)	**Cortical stroke (n=46)**	**p value**
Male sex, n (%)	21 (52.5%)	39 (84.7%)	0.002 **
Age, mean (SD) years	66.5 (11.3)	71.1 (10.9)	0.055
Hypertension, n (%)	25 (63%)	29 (63%)	1.000
Diabetes, n (%)	9 (22.5%)	5 (10.8%)	0.244
IHD, n (%)	7 (17.5%)	12 (26.0%)	0.486
Atrial fibrillation, n (%)	2 (5%)	8 (17.4%)	0.147
Hyperlipidemia, n (%)	15 (37.5%)	17 (36.9%)	1.000
Total cholesterol, mean (sd) mmol/l	5.26 (1.23) (n=35)	5.02 (1.14) (n=41)	0.382
Positive family history of stroke, n (%)	5 (12.5%)	3 (6.5%) (n=45)	0.584
Inflammation			
CRP, median (Q1 – Q3) mg/L	1.36 (0.74 – 3.10)	1.68 (0.84 – 3.25)	0.703
TNF-α, median (Q1 – Q3) pg/mL	0.92 (0.72 – 1.37)	0.85 (0.76 – 1.37)	0.849
IL-6, median (Q1 – Q3) pg/mL	2.57 (1.77 – 4.26)	2.54 (1.81 – 3.57)	0.962
Endothelial dysfunction			
ICAM, mean (SD) ng/mL	155.03 (55.66)	158.93 (45.39) (n=44)	0.727
vWF, mean (SD) iu/dL	131.8 (43.72)	133.9 (42.1)	0.822
Thrombosis			
Fibrinogen, mean (SD) g/L	3.70 (0.59)	3.84 (0.61) (n=45)	0.478
t-PA, mean (SD) ng/mL	6.94 (3.15)	8.29 (2.87)	0.042 *
D-dimer, median (Q1 – Q3) ng/mL	91.5 (69 - 152)	119.5 (71 – 165)	0.828

* p<0.05. **p<0.01. CRP = C-reactive protein, ICAM = intracellular adhesion molecule-1, IL-6 = interleukin-6, NIHSS = National Institute Health Stroke Scale, TNF-α = tumour necrosis factor alpha, t-PA = tissue plasminogen activator, vWF = von Willebrand factor.

Meta-analysis

On addition of our study to the four prior studies (new total 300 lacunar strokes), we show lower t-PA in lacunar versus non-lacunar stroke although the difference was not significant (Figure 3.1). Addition of this study's data moves the standardised mean difference from 0.02 to (95% CI -0.18 to 0.21) to -0.08 (-0.25 to 0.09).

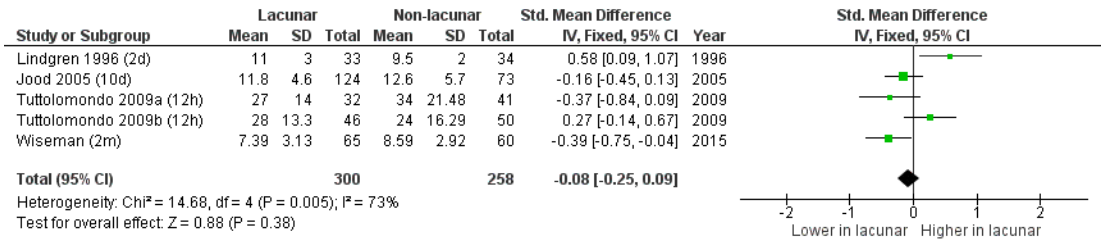


Figure 3.1 Forest plot comparing t-PA levels between lacunar stroke and non-lacunar stroke. Values in bracket after Study refers to time to blood draw.

Biomarkers and WMH (all patients, lacunar and cortical)

Quantitative WMH and plasma biomarkers were available for 98 patients. The baseline model (age, sex, hypertension and smoking status) explained 29% of the variance in quantitative WMH (RSE 1.081, R^2 0.289) with age, hypertension and smoking as significant predictors (Table 3.8).

Model 1 (baseline + INF) showed minor improvement over baseline (RSE 1.066, R^2 0.291). Models 2 (END), 3 (THR) and 4 (INF+END+THR) did not improve the baseline model. All models met model assumptions. There were no negative correlations between the components of the summed variables meaning a rise in one plasma marker was not offset by a fall in another.

Table 3.8 Explaining variance in quantitative WMH with different predictor variables (n=98)

Model	Predictor variables	RSE	R^2
Baseline	Age***, male sex, hypertension*, smoking**	1.081	0.289
Model 1	Age***, male sex, hypertension*, smoking** + *Inflammation*	***1.066***	***0.291***
Model 2	Age***, male sex, hypertension*, smoking** + *Endothelial activation*	1.098	0.285
Model 3	Age***, male sex, hypertension*, smoking** + *Thrombosis*	1.098	0.278
Model 4	Age***, male sex, hypertension*, smoking** + *Inflammation + Endothelial activation + Thrombosis*	1.094	0.285

* p<0.05, ** p<0.01, *** p<0.001.
Inflammation = $_{log}$CRP, $_{log}$TNF-α, $_{log}$IL-6
Endothelial activation = vWF, ICAM
Thrombosis = t-PA, $_{log}$D-dimer, fibrinogen
RSE = residual standard error (***bold*** = improvement over baseline, ie, reduction in RSE)
R^2 = adjusted R-squared (***bold*** = improvement over baseline, ie, increase in R^2)

Discussion

We show a difference in t-PA levels between lacunar stroke and mild cortical stroke from plasma sampled well after the acute phase, independent of age, sex and risk factors. We did not find differences between stroke subtypes for biomarkers of inflammation (CRP, TNF-α or IL-6), endothelial dysfunction (vWF or ICAM) or other markers of haemostasis (fibrinogen or D-dimer). Except for a minor additional predictive effect of summed inflammatory markers, plasma biomarkers did not considerably improve the baseline model in explaining WMH.

t-PA

t-PA is a glycoprotein released mainly by endothelial cells[143,144] to mediate the breakdown of thrombus. Its use as a thrombolytic agent might lead one to assume endogenous t-PA is protective against thrombosis[144]. However, higher t-PA antigen levels are associated with risk of coronary heart disease in generally healthy populations[143]. This may reflect increased endothelial disturbance resulting in increased t-PA secretion; or else increased levels of its inhibitor, tissue plasminogen activator inhibitor (PAI), resulting in increased levels of circulating complexes with t-PA[143–145]. The effect of recombinant t-PA subdivided by smoking status at baseline was not investigated in the IST-3[146] trial although some[147,148] but not all[149] observational studies suggest that smokers respond better to recombinant t-PA than non-smokers when treated with intravenous recombinant t-PA for acute ischaemic stroke.

We found lower t-PA in lacunar versus cortical stroke. Reduced t-PA could mean lacunar stroke patients have reduced vascular damage – vWF levels were also lower

in lacunar stroke but ICAM levels were higher (neither statistically significant). Alternatively lacunar stroke patients might have increased endogenous fibrinolytic activity, if lower t-PA levels reflect lower levels of its inhibitor, PAI.

Knottnerus et al.[150] found significantly lower t-PA levels (and significantly higher PAI levels) in 43 lacunar stroke patients with an isolated infarct versus 53 lacunar stroke patients with concurrent extensive WMH, hypothesizing that patients with extensive WMH lack the protective effect of PAI for t-PA induced tissue damage.

In our recent meta-analysis (Chapter 2)[127], t-PA was significantly higher in lacunar stroke versus non-stroke controls but did not differ significantly between lacunar stroke and other stroke subtypes, although data were limited and the timing of sample collection could be confounding. The samples in the current study were collected well after the acute phase and are more likely to reflect underlying pathway activity. The updated meta-analysis including the current data, moves the evidence in favour of lower t-PA in lacunar versus non-lacunar stroke (Figure 3.1). The largest study to date to find lower levels of t-PA in small vessel stroke is the Sahlgrenska cohort, Sweden[92]: among 600 patients with ischaemic stroke, including 124 with small vessel stroke, small vessel stroke patients had higher t-PA levels compared to non-stroke controls in the acute phase and at three months but lower t-PA levels compared to other stroke subtypes.

The lacunar group were significantly younger with fewer cases of atrial fibrillation and more smokers (nonsignificant) than the cortical group, although the association of lacunar stroke with lower t-PA was independent of these and the pattern persisted in analysis restricted to non-smokers.

WMH and biomarkers

Age, hypertension and smoking were significant predictors of WMH. The inflammatory biomarker summed variable appeared to improve the model (slight reduction in the residual standard error) but the additional explanatory power was small and could be interpreted as no model improvement. On the other hand, a similar effect size confirmed in a larger study would indicate a modest but important effect of plasma markers of inflammation on WMH prediction. The other plasma markers did not have any additional explanatory power. Studies that have measured WMH in non-stroke populations typically involve older people. We have clearly shown age to be the most important predictor variable in the assessment of WMH and thus correcting for age is crucial.

Two large studies[67,128] showed independent associations between higher plasma inflammatory biomarkers and more WMH but had wide age ranges. However, Rouhl et al.[71] found no difference in CRP levels between 81 patients with and 265 patients without extensive WMH, Wersching et al.[129] found no association between CRP and WMH among 321 older stroke-free participants, Baune et al.[151] found no association between TNF-α and WMH among 268 community-dwelling participants and Aribisala et al.[48] found no association between inflammation (a latent factor comprising CRP, fibrinogen and IL-6) and WMH among 634 community-dwelling older people of near-identical age. Thus it is possible that wide age ranges in some studies inflated associations between inflammatory markers and WMH. Shoamanesh et al.[131] found no association between some inflammatory biomarkers (including CRP, IL-6 and TNF-α) and SVD (defined as presence of silent infarcts and/or extensive WMH) in a

large cohort of younger stroke-free Framingham participants (n=522; mean age 60 years) but did associate ICAM with SVD. We found no association between ICAM and WMH in the present study but have much less power than the Framingham study. Our systematic review and meta-analysis (Chapter 2)[127] found no difference in ICAM levels between lacunar stroke and other stroke subtypes although only a few studies contributed data. ICAM was non-significantly higher in lacunar stroke versus cortical stroke patients in the present study.

Conclusion

Our findings show a difference in t-PA levels between lacunar and cortical stroke which should be verified in other datasets. Future studies should obtain plasma samples in the chronic phase after stroke and concentrate on longitudinal associations, especially the role of t-PA in stroke subtypes as it could help explain mechanisms. A large prospective study of accurately phenotyped stroke patients would be helpful. It is important to control for age specifically, but also hypertension and smoking when modelling features of SVD such as WMH and biomarkers.

Chapter 4: Cerebrovascular disease in rheumatic diseases: A systematic review and meta-analysis

[now published: Wiseman et al. *Stroke.* 2016;47:943–950]

Introduction

Stroke is a major health problem. Overall incidence rates are falling[152,153] but better access to medical care and improvements in secondary prevention increase survival so stroke prevalence, and thus healthcare costs, remain high. An ageing population will increase this trend.

Rheumatic diseases such as RA are an independent risk factor for stroke[154,155]. People with these diseases die prematurely from cardiovascular disease including stroke,[156,157] so a deeper understanding of stroke risk among these patients is needed to reduce mortality. However, data linking rheumatic diseases with higher stroke risk are based mostly on stroke reported from large population studies, ie, a composite outcome of *any* stroke. Less is known about associations between rheumatic diseases (inflammatory or non-inflammatory) and major stroke subtypes whose mechanisms differ, e.g. ischaemic versus haemorrhagic stroke, or large artery atheromatous versus intrinsic small vessel ischaemic stroke, or with conditions associated with cerebral small vessel disease (SVD) such as cognitive decline, mood change and gait disturbances[16,18].

Population stroke incidence rises with age. Stroke early in life is rare among the general population, yet most of the stroke associated with rheumatic diseases appears to be at younger ages,[158–164] and may level off, as some studies[161,162,165,166] report no

risk difference in those over 65 years. However, there is currently no meta-analysis on the overall association of rheumatic diseases with stroke by age. Clarifying timing of greatest stroke risk has important clinical implications.

Studies to date do not fully explain the increased stroke risk among rheumatic populations by vascular risk factors[167,168]. Some stroke risk in rheumatic diseases could relate to the higher inflammatory activity seen in many arthropathies which is systemic, non-resolving and often only controlled with aggressive anti-rheumatic drugs. Inflammation therefore plausibly explains some of the excess risk due to atheromatous stroke as inflammation is involved in all stages of atherosclerosis from fatty streak formation to plaque disruption[43–45]. The role of inflammation in SVD is less certain but inflammation is seen pathologically in the perforating arteriolar walls and perivascular tissue[46,47]. Endothelial damage is a primary step in atherosclerosis and SVD and factors that contribute to endothelial damage (eg, immune complex formation and complement activation/deposition) are also seen in rheumatic diseases.

The aims are to review associations between stroke and rheumatic disease; to summarise incidence rates and calculate pooled rate ratios for stroke subtypes versus the general population; to see if risk is greatest at specific ages; and to determine if rheumatic diseases increase the risk of 'silent' vascular disease on neuroimaging.

Methods

Study design

We used a systematic approach to assess stroke and stroke subtypes as the outcome measure and various rheumatic diseases as the exposure. Research ethics committee approval was not required. The study was not registered in any database.

Data sources

The review was prepared in accordance with the *Preferred reporting items for systematic reviews and meta-analyses* (PRISMA) statement[75]. Structured search terms (Table 4.1) were used to query EMBASE (from 1980) and MEDLINE (from inception) to 2014 on 14 December 2014. Data were extracted in accordance with *Meta-analysis of observational studies* (MOOSE) guidelines[76]. We categorized magnetic resonance imaging (MRI) findings according to STRIVE guidelines[19].

Table 4.1 Search strategy

1. brain ischemia/ or brain infarction/ or brain stem infarctions/ or cerebral infarction/ or hypoxia-ischemia, brain/ or stroke/
2. (isch?emi$ adj6 (stroke$ or apoplex$ or cerebral vasc$ or cerebrovasc$ or cva or attack$)).tw.
3. ((brain or cerebr$ or cerebell$ or vertebrobasil$ or hemispher$ or intracran$ or intracerebral or infratentorial or supratentorial or middle cerebr$ or mca$ or anterior circulation) adj5 (isch?emi$ or infarct$ or thrombo$ or emboli$ or occlus$ or hypoxi$)).tw.
4. 1 or 2 or 3
5. Arthritis/ or Arthritis, Rheumatoid/ or Autoimmune Diseases/ or Musculoskeletal Diseases/ or Rheumatic Diseases/
6. 4 and 5

Study selection

English language studies reporting on stroke in rheumatic disease and studies that assessed brain imaging features of SVD on MRI were included. Studies using functional MRI, positron emission tomography, single photon emission computed tomography and Doppler ultrasound were excluded.

Data extraction

Data on study population demographics, control groups, stroke type (ischaemic / haemorrhagic), ischaemic stroke subtypes (large artery / lacunar), findings on stroke risk relative to a comparator group and MRI findings were extracted. Stroke incidence rises with age, and we noted if studies controlled for age and vascular risk factors.

Quality assessment

A quality checklist adapted from the *Strengthening the reporting of observational studies in epidemiology* (STROBE) statement[79] was used to assess the quality of included studies (Table 4.2).

Table 4.2 Quality checklist for included studies

Prospective or retrospective?
Was stroke subtyped: ischaemic / haemorrhagic / large artery / lacunar?
Was criteria/system used to subtype stroke well explained?
Was neuroimaging used to confirm stroke diagnosis? Was diffusion-weighted imaging used?
Did authors adequately describe and/or rule out stroke mimics?
Did authors provide number of stroke events and number of person-years observation?
Was the comparison group well described (including number of stroke events and number of person-years observation)?
Did the study match comparators on age, sex and traditional vascular risk factors (hypertension, diabetes, hyperlipidaemia) or control for these at the analysis stage?

Data synthesis and analysis

We defined SVD from MRI features as per STRIVE neuroimaging standards[19], being any of: recent small sub-cortical infarcts, white matter hyperintensities (WMH), lacunes, microbleeds, prominent peri-vascular spaces (PVS) or atrophy. Clinically, patients might show no symptoms, or they might suffer cognitive impairment or other neurological involvement in addition to stroke (lacunar stroke accounts for around 25% of all ischaemic strokes[169]).

We defined stroke incidence rates as number of strokes as a function of a follow-up period and stroke rate ratios as the ratio of stroke incidence rate in the observed group (eg, rheumatoid arthritis) over incidence rates in the general population. We used unadjusted (crude) rates throughout as different studies controlled for different variables making comparison of uniform adjustments impossible.

Stroke incidence rates were recorded when reported and calculated when not reported but where data (ie, number of stroke events and a follow-up period) were available. All incidence rates were converted to "per 100,000 person-years" for comparability. If follow-up duration (in patient-years) was not specifically reported, we estimated this by multiplying number of patients by the average years of follow-up.

Where a rheumatic disease had contributing data from more than one study, we pooled incidence rates by taking the range of available values, which did not allow us to assess heterogeneity. Next, we calculated a point estimate for incidence rate per 100,000 person-years for individual rheumatic diseases based on the weighted mean, using study size as the weighting factor and then estimated a 95% CI based on the Poisson

distribution as implemented in the epitools package for the statistical programming language R version 3.0.1 (http://www.r-project.org/)[141].

Rate ratios were calculated using the Cochrane Collaboration's Review Manager 5 software when not reported but where required data were available.

Some studies did not provided number of strokes and number of patient-years observed for the control group but instead only provided the rate ratio together with a CI. As per Cochrane Handbook[84], CIs can be converted to standard errors and the natural logarithms of rate ratios may be combined across studies using the generic inverse-variance method. We used this approach to pool stroke risk for ischaemic stroke and haemorrhagic stroke, and for the age category pooled analysis.

Between-study heterogeneity was assessed using the I^2 statistic. We used random effects models in all meta-analyses.

Results

The search returned 443 titles and abstracts; 69 papers were reviewed in full and 23 studies contributed data to new meta-analyses. We excluded studies that did not measure stroke with appropriate imaging (n=12), pathology studies (n=5), guidelines and review papers (n=4) and small studies (<50 patients) that only described imaging features of SVD (n=25) (Figure 4.1).

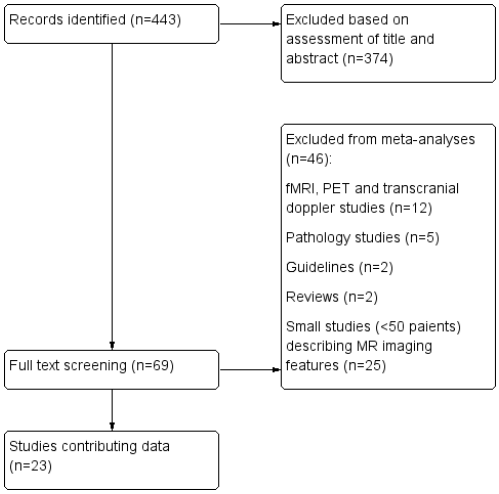


Figure 4.1 Summary of search and selection

Any stroke

Prior meta-analyses and large registry studies of any stroke are reported in Table 4.3. Briefly: RA (prototypical inflammatory rheumatic disease) showed significant risk of stroke over the general population (n=26,143 patients; incidence rate ratio (RR) 1.91, 1.73 to 2.12)[170] while osteoarthritis (degenerative) did not (n=40,817 patients; odds ratio (OR) 1.11, 0.95 to 1.29)[171]. In direct comparison, patients with osteoarthritis alone (no comorbid RA) had lower stroke risk (11,633 RA versus 163,274 OA patients; OR 1.3, 1.2 to 1.3, i.e., higher stroke risk in RA)[172]. Ankylosing spondylitis

(OR 1.51, 1.39 to 1.62) and gout (RR 1.71, 1.68 to 1.75) also showed higher risk of stroke than the general population.

Table 4.3 Summary of prior (and new) stroke risk findings – *any* stroke

Rheumatic disease	**Stroke in general**
RA	Meune et al.[170] meta-analysed stroke death among nine studies involving 88,500 rheumatoid arthritis (RA) patients and stroke incidence among three studies involving 26,143 RA patients: risk of death (standardised mortality ratio 1.46, 95% CI 1.31–1.63) and stroke incidence (incidence rate ratio 1.91, 95% CI 1.73–2.12) were higher than the general population, respectively. Lindhardsen et al.[158] linked Danish national registers to follow 18,247 RA patients over 13 years. Stroke incidence in RA was significantly higher than in the general population (rate ratio 1.33, 95% CI 1.23–1.43).
SLE	New meta-analysis (See text and Figure 4.2). Included studies[162,166,173–175].
AS	Meta-analysis[176] of three studies of 9,791 ankylosing spondylitis (AS) patients found an increased risk of stroke versus the general population (odds ratio (OR) 1.51, 95% CI 1.39–1.62). Two subsequent studies (n=5,000 AS patients)[177,178] not included in the meta-analysis show similar risk of stroke (hazards ratio 1.2 (1.0–1.5) and 2.3 (1.9–2.8)) over the general population, while a third study[179] reported an increased risk of vascular death (a composite end point of heart attack and stroke) among n=21,473 patients of 1.36 (1.13–1.65) versus healthy controls.
Gout	Gout patients have (rate ratio 1.71 (95% CI 1.68–1.75)) times the risk of stroke over the general population from a study of 767,725 person-years follow-up[164].
Psoriasis and PsA	New meta-analysis (See text and Figure 4.3). Included studies[180–191]. Additionally, Chin et al.[192] compared psoriasis patients (n=383 strokes; n=7,397 patients) to PsA patients (n=23 strokes; n=225 patients) in a population-based retrospective cohort study and found a non-significant increase in stroke risk in PsA patients (hazard ratio 1.5, 95% CI 0.98–2.29) which became significant on multivariate modelling controlling for age, hypertension diabetes, dyslipidaemia and phototherapy (HR 1.82, 95% CI 1.17–2.82).
OA	Compared to RA, patients with osteoarthritis (OA) alone (eg, no comorbid RA) are at lower risk of stroke (11,633 RA patients versus 163,274 OA patients; rate ratio 1.3, 95% CI 1.2–1.3), ie, RA has higher risk[172]. A more recent study[171] of 40,817 patients with OA concluded there was no additional stroke risk in OA versus the general population, although the strokes were self-reported.
AS = ankylosing spondylitis, OA = osteoarthritis, PsA = psoriatic arthritis, RA = rheumatoid arthritis, SLE = systemic lupus erythematosus	

We add new meta-analyses on any stroke in SLE, psoriasis and psoriatic arthritis. The pooled odds of any stroke in SLE from five studies[162,166,173–175] (772 strokes, 40,652 SLE patients) was 2.13 (1.53 to 2.98) (Figure 4.2).

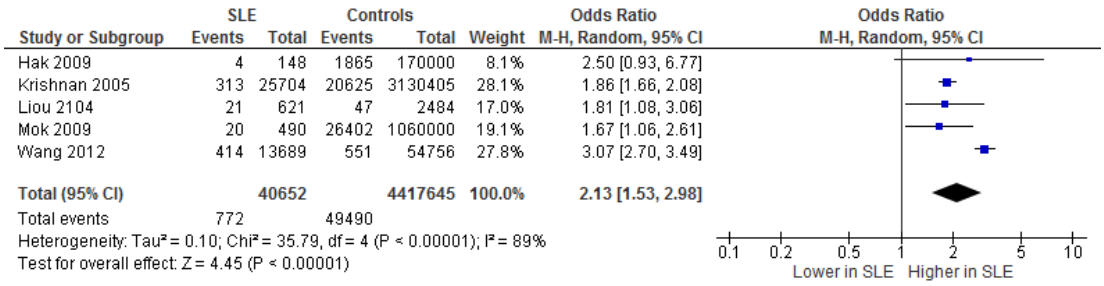


Figure 4.2 Any stroke in systemic lupus erythematosus

We updated two psoriasis meta-analyses[193,194] and added a new meta-analysis for psoriatic arthritis. The pooled odds of any stroke in psoriasis (nine studies,[180–186,190,191] 6,925 strokes; 400,767 patients) was 1.08 (1.00 to 1.16) and psoriatic arthritis (three studies[180,181,187] 217 strokes; 12,051 patients) was 1.27 (0.98 to 1.64) (Figure 4.3).

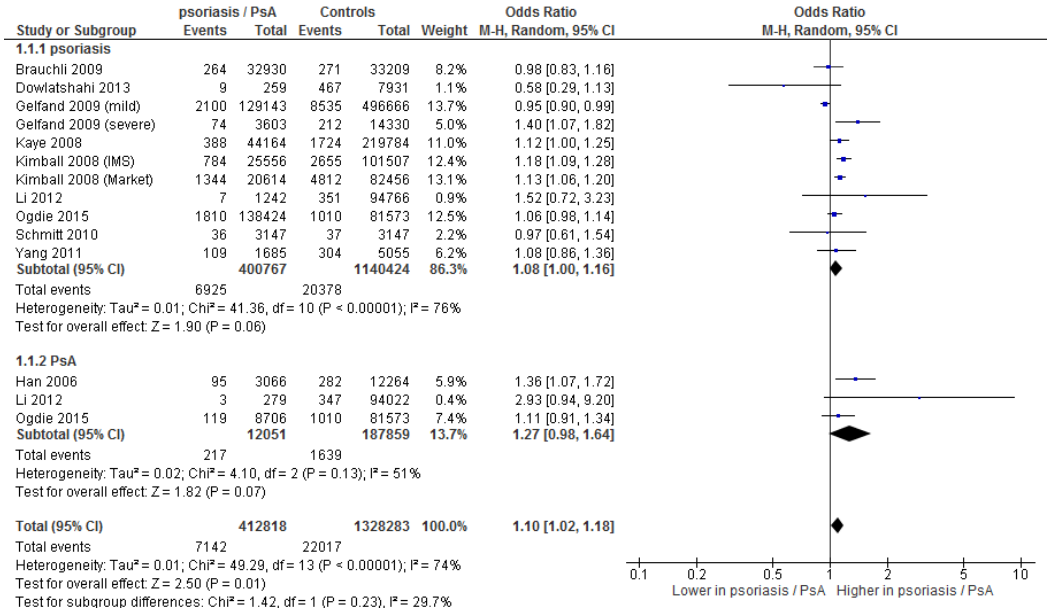


Figure 4.3 Any stroke in psoriasis and psoriatic arthritis (PsA)

Stroke subtypes: ischaemic and haemorrhagic

Stroke ***incidence*** of ischaemic and haemorrhagic stroke by rheumatic diseases is summarised in Table 4.4.

Table 4.4 Stroke incidence rates by stroke subtype among different rheumatic diseases

Rheumatic disease	Included studies	Strokes	Number of person-years follow-up	Range of incident rates, per 100,000 person-years	Mean IR, per 100,000 person-years (95% CI)	References
Ischaemic						
Rheumatoid Arthritis	6	3,611	1,193,249	178 to 1,077	303 (269–337)	163,178,195–198
Gout	1	5,391	767,725	702	702 (650–754)	164
Ankylosing Spondylitis	1	111	76,494	145	145 (121–169)	178
Reiter's	1	13	7,436	175	175 (149–201)	178
Psoriasis / PsA	None					
Polyarteritis Nodosa	1	46	18,106	254	254 (223–285)	178
Ploymyalgia	1	1,777	362,912	489	489 (446–532)	178
SLE	7	995	271,076	208 to 2,530	367 (329–404)	162,166,174,178,199–201
Scleroderma	1	44	11,264	391	391 (352–430)	178
Sjogren's	1	68	28,600	238	238 (208–268)	178
Osteoarthritis	None					
General population					*141 (127–156)*	202
Haemorrhagic						
Rheumatoid Arthritis	3	562	1,096,594	43 to 118	51 (37–65)	163,178,196
Gout	1	1,864	767,725	242	242 (211–272)	164
Ankylosing Spondylitis	1	42	76,494	55	55 (40–69)	178
Reiter's	1	2	7,436	27	27 (17–37)	178
Psoriasis / PsA	None					
Polyarteritis Nodosa	1	5	18,106	28	28 (18–38)	178
Polymyalgia	1	204	362,912	56	56 (41–71)	178
SLE	4	164	223,027	35 to 118	74 (57–91)	162,166,174,178
Scleroderma	1	8	11,264	71	71 (54–87)	178
Sjogren's	1	5	28,600	17	17 (9–25)	178
Osteoarthritis	None					
General population					*12 (9–17)*	202

PsA = psoriatic arthritis, SLE = systemic lupus erythematosus. Mean IR = Mean incident rate based on a weighted mean (weight based on study size, ie, person-years observed)

Sufficient data to perform meta-analysis of ***stroke risk (rate ratios)*** among stroke subtypes versus the general population were only available for RA and SLE. In RA versus the general population, the pooled odds of ischaemic stroke (3,481 strokes; 86,280 patients) and haemorrhagic stroke (562 strokes; 84,419 patients) were 1.64

(95% CI 1.32 to 2.05) and 1.68 (1.11 to 2.53) respectively (Figures 4.4 and 4.5). In SLE versus the general population, the pooled odds of ischaemic stroke (945 strokes; 55,699 patients) and haemorrhagic stroke (164 strokes; 44,062 patients) were 2.11 (1.66 to 2.67) and 1.82 (1.07 to 3.09) respectively (Figures 4.4 and 4.5).

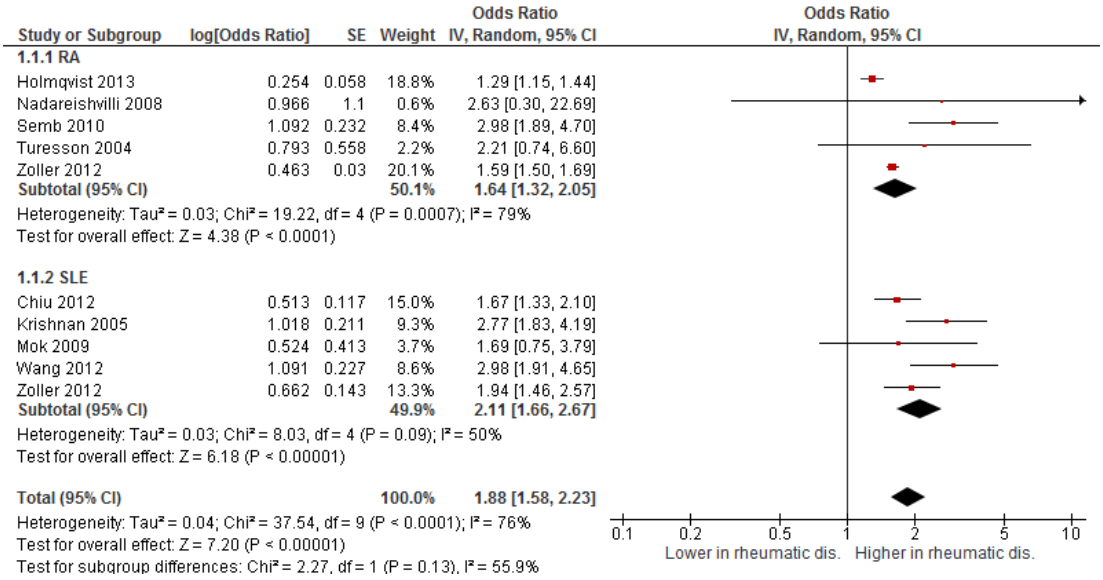


Figure 4.4 Forest plot – Ischaemic stroke in rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) versus general population

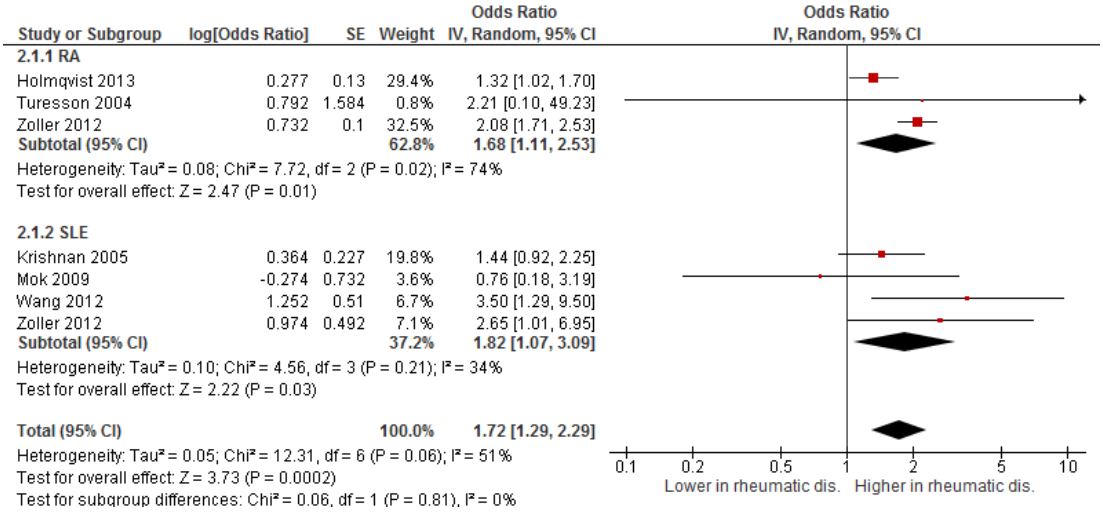


Figure 4.5 Forest plot – Haemorrhagic stroke in rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) versus general population

Subtypes of ischaemic stroke

For SLE, two studies[166,199] provide incidence rate data for *ischaemic* stroke subtypes: among 490 SLE patients[166], 13 had cortical strokes (equivalent to 271 cortical strokes per 100,000 person-years) and four had lacunar strokes (83 per 100,000) from 4,802 person-years follow-up; among 232 SLE patients[199], 20 had large artery strokes (1,150 per 100,000), 17 had small vessel strokes (997 per 100,000) and four had cardioembolic strokes (230 per 100,000) from 1,739 person-years follow-up. There were limited comparative data from the general population but Sacco et al.[203] report annual incidence rates for lacunar stroke as 33 per 100,000 population. There were insufficient data to pool rate ratios among ischaemic stroke subtypes.

Age, rheumatic disease and stroke

The pooled odds of any stroke (11,879 strokes; 340,548 patients)[158,161–166,174,189] across five rheumatic diseases (data were available for RA, SLE, psoriasis, ankylosing spondylitis and gout) versus the general population was 1.38 (95% CI 1.21 to 1.57) (Figure 4.6). When split by age, the pooled odds were 1.79 (1.46 to 2.20) for age <50 years, 1.49 (1.07 to 2.06) for age 50-65 and 1.14 (0.94 to 1.38) for age 65 and above (Figure 4.6). The age categories were significantly different (χ^2 test for subgroups, p=0.007). A study-by-study review of stroke by age categories across stroke in general and by ischaemic and haemorrhagic stroke subtypes is given in Table 4.5.

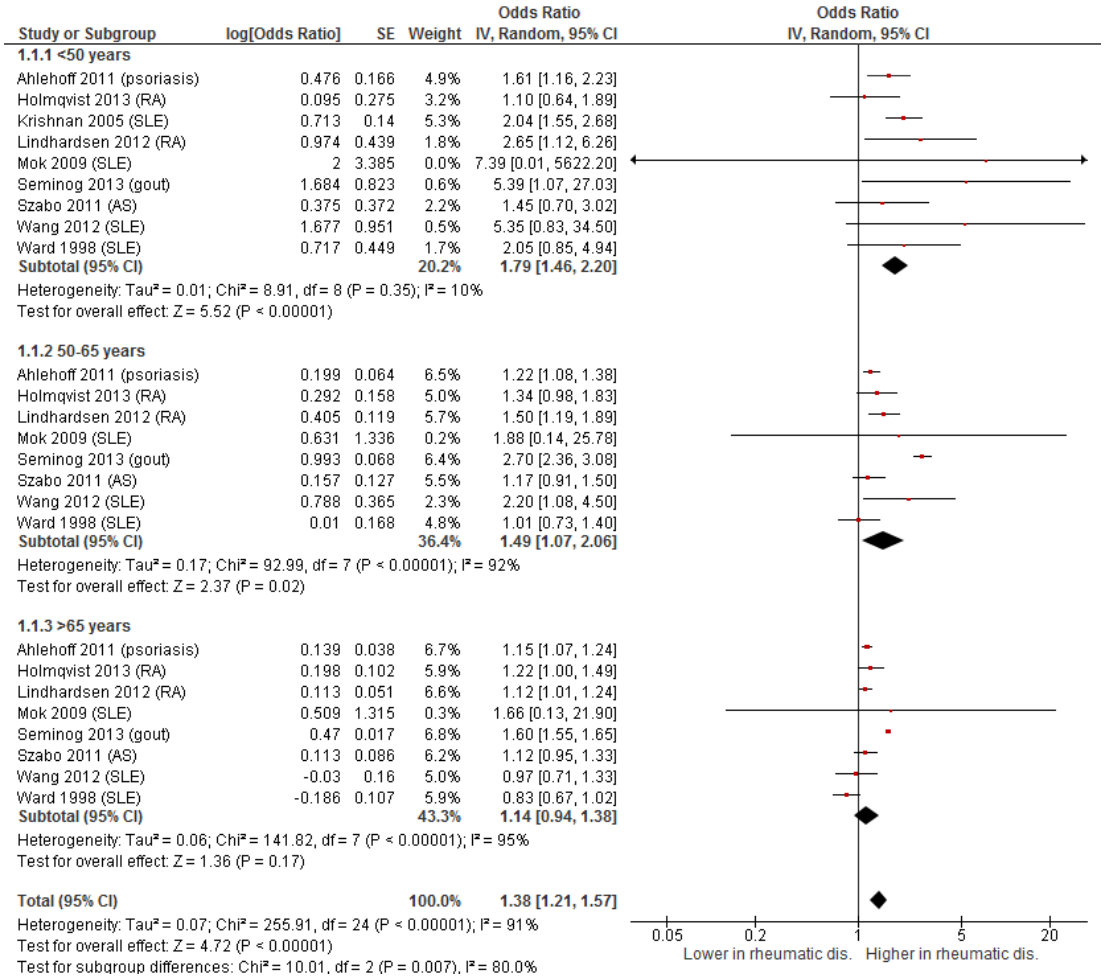


Figure 4.6 Forest plot – Any stroke in rheumatic diseases by three age categories

Table 4.5 Summary of stroke risk findings by age: stroke in general and by subtype

Stroke type	Finding
Any stroke	Lindhardsen et al.[158] report a rate ratio for stroke among male RA patients versus the general population as 3.61 (95% CI 2.05–6.36) for those aged < 50 years, 1.70 (1.34–2.15) for those aged 50-65 years and 1.21 (1.05–1.40) for those aged 65 years and older. A similar trend was seen among women. Solomon et al.[159] report a rate ratio for any cardiovascular event including stroke among 18-49 year-old RA patients versus the general population as 3.3 (95% CI 2.4–4.5), 2.2 (1.9–2.5) for those aged 50-64 years, 1.9 (1.7 to 2.1) for those aged 65-74 years and 1.6 (1.5–1.7) for those aged 75 years and older.

	Ward[161] reports twice as many strokes among 18-44 year old women with SLE compared with the general population (rate ratio 2.05; 95% CI 1.17–2.93) whereas in those aged 45-64 years and ≥ 65 years there was no such significant increase in stroke rates. Among 13,689 SLE patients followed for over 5 years, Wang et al.[162] found stroke risk relative to the general population to diminish over the lifecourse. Hazards ratios were: 31.1 (95% CI 14.2–68.3) in 18-24 year olds, 15.1 (9.3–24.5) in 25-34 year olds, 5.3 (3.8–7.5) in 35-44 year olds, 2.3 (1.7–3.1) in 45-54 year olds, 2.2 (1.6–3.0) in 55-64 year olds with no risk difference in those 65 years and older (0.97, 0.7–1.3). Bessant et al.[160] assessed stroke risk in 202 SLE patients (92% female; mean age 42 years) using software (with Framingham data) against hypothetical age- and sex-matched controls. Patients had a higher 10-year risk of stroke versus control data ($p<0.0001$). When stratified by age, patients under 40 years continued to be at significantly higher risk but patients over 40 years showed no difference to controls. Szabo et al.[165] found the excess risk for stroke in ankylosing spondylitis (AS) over the general population was highest among younger AS patients (example: females aged 20-39 years had a risk ratio of 1.69 (95% CI 1.23–2.33) while females aged 40-59 years and ≥60 years had non-significant risk ratios of 1.15 and 1.14 respectively). Ahlehoff et al.[189] report reducing stroke risk with increasing age in mild psoriasis over the general population: 18-50 years (1.61 (95% CI 1.32–1.97)), 51-70 years (1.22 (95% CI 1.10–1.35)) and >70 years (1.15 (95% CI 1.05–1.20)).
Ischaemic and haemorrhagic subtype	Among prevalent RA, Holmqvist et al.[163] report a hazard ratio for ischaemic stroke versus the general population to be 0.81 (95% CI 0.43–1.54) for those aged 16–52 years, 1.47 (1.13–1.90) for those aged 53–62 years, 1.36 (1.14–1.62) for those aged 63–71 years and 1.25 (1.12–1.41) for those aged 72–94 years. The hazard ratios for haemorrhagic stroke among the same age bands were: 1.76 (0.85–3.63), 1.00 (0.61–1.65), 0.82 (0.53–1.25) and 1.75 (1.34–2.29) respectively. Mok et al.[166] report significantly higher levels of ischaemic stroke in young SLE patients (in each 10-year age band up to 50 years) versus the general population, but the difference becomes non-significant in each 10-year age band in those over 50 years. Young SLE patients (below 40 years) had significantly higher rates of haemorrhagic stroke versus the general population, but data were limited with no cases of haemorrhagic stroke in those over 40 years. From 25,704 SLE patients hospitalised in the US between 2001–2002, Krishnan[174] found 313 strokes (206 as the primary diagnosis) giving an age- and sex-adjusted risk estimate for stroke in young (< 50 years) SLE patients of 1.5 (95% CI 1.3–1.8). SLE patients had higher stroke risk for all stroke subtypes except in subarachnoid haemorrhage where a trend to lower risk was observed (OR 0.57, 95% CI 0.34–0.96). The study was limited to those aged < 50 years. Seminog and Goldacre[164] report the relative risk for ischaemic stroke among gout patients versus the general population as 4.68 (95% CI 2.89–7.18) in those aged 20–44 years, 2.68 (95% CI 2.51–2.86) in those aged 45–69 years and 1.56 (95% CI 1.51–1.61) in those over 70 years. The relative risk for haemorrhagic stroke was 7.49 (95% CI 4.67–11.40) in those aged 20–44 years, 3.02 (95% CI 2.72–3.34) in those aged 45–69 years and 1.49 (95% CI 1.41–1.58) in those over 70 years.

Structural MRI brain imaging findings

Numerous MRI studies of the brain in rheumatic diseases were reviewed. Most were small, many investigated neurologically symptomatic patients only (e.g., comparing neurolupus with SLE), and few controlled for age and known vascular risk factors.

Of the larger studies (>50 patients) that provided information on features of SVD, there were consistent reports of WMH and brain atrophy in rheumatic disease patients (n=13 studies (11 in SLE) involving 1,411 patients) (Table 4.6)[61,62,204–214].

However, only five studies[61,62,205,206,209] compared rheumatic disease patients (n=414 patients) to non-rheumatic healthy controls, and three of these were from the same research group and so only ~224 patients contribute data versus healthy controls. Two studies report on WMH: Hamed et al.[62] found no difference in WMH between 55 patients with RA and 40 healthy controls, although they excluded seven patients with white matter disease before comparing the remaining 48 patients, and they did not report on whether a grading system or a volumetric calculation was used to assess the WMHs. Harboe et al.[61] report more WMH in 62 patients with SLE versus 62 age- and sex-matched healthy controls (6.0 *v* 4.1, p=0.05; Scheltens' score).

Longitudinal data were also limited. Appenzeller et al.[206] followed 75 SLE patients over almost two years and found significant brain atrophy particularly in the corpus callosum (baseline versus follow-up scan, p=0.001). Additionally, predictors of new or increased WMH included antiphospholipid antibodies, SLE damage scores and higher dose of corticosteroids[207].

Table 4.6 Summary of structural MRI brain imaging findings in rheumatic diseases

Study	Disease	N	Mean age (years)	Findings
Kaichi, 2013[204]	SLE	256	39.0	Significantly more patients with APS had lacunar infarcts in the deep white matter (p<0.01) (but not the basal ganglia), cortical infarcts in the MCA territory (p<0.01), bilateral borderzone infarcts (p<0.01) and basal ganglia lesions (p=0.01) versus SLE patients without APS. WMH (rated with Fazekas) did not differ between SLE patients with and without APS.
Steup-Beekman, 2013[211]	SLE	155	27.5	Among n=102 with NPSLE, 47% had a normal MRI. WMH were found in 31/102 (30%) and atrophy in 20/102 (20%).
Akasbi, 2012[210]	SS	51		Dichotomised 51 Sjögren's Syndrome patients into those with WMH (n=25) and those without (n=26) using Wahlund scale. Those with WMH were older (70.3 v 58.3; p=0.004) and had higher frequency of cardiovascular risk factors.
Hamed, 2012[62]	RA	55	45.6	Removed 7 patients with probable white matter disease, then compared n=48 patients with 40 healthy controls and found no difference in T2 or FLAIR 'hyperintense signals' (p=0.245). Unclear how the hyperintensities were measured.
Katsumata, 2010[212]	SLE	191	32.0	Compared those with (n=57) and without (n=134) neurolupus. Abnormal MRI were more often found in those with neurolupus (RR 1.7, 95% CI 1.1–2.7). Large abnormal signals (>10mm) were only seen in the neurolupus group (n=7) whereas small abnormal signals were seen in both groups.
Harboe, 2008[61]	SLE	62	44.3	SLE patients have more fatigue (p<0.0001) and WMH compared with healthy controls (p=0.05). Total WMH increased more with age in patients than in controls.
Valdes-Ferrer, 2008[208]	SLE	71	32	Compared SLE patients with and without APS. WMH were found in more than 40% of patients from both groups (non-significant).
Appenzeller, 2008[207]	SLE	120 (80 with repeat MRI)	33.3	At baseline, 50% of patients had WMH: mean volume 197 mm3 (FLAIR images). WMH were associated with age (p=0.01), total corticosteroid dose (p=0.001) and damage from SLE (p=0.002). Predictors for new or increased WMH at follow-up (median 24 months) were past CNS involvement, antiphospholipid antibodies, SLE damage score and higher dose of corticosteroid dose.
Appenzeller, 2007[206]	SLE	75	32.3	SLE patients have reduced white and grey matter volumes versus healthy controls (p=0.001). On follow-up, there is progressive white and grey matter atrophy in patients (p=0.001)
Appenzeller, 2006[209]	SLE	107	32.2	SLE patients have smaller hippocampal volumes versus controls (p<0.001). At follow-up (n=60 patients, mean 19 months) there was significant reduction in hippocampal volume over baseline volumes. Cognitive impairment was associated with hippocampal volume loss (r=0.89; p=0.001).

Appenzeller, 2005[205]	SLE	115	33.5	Cerebral and corpus callosum volumes were significantly smaller in SLE patients versus controls ($p<0.001$). Patients with cognitive impairment had significantly reduced cerebral and corpus callosum volumes compared with SLE patients without cognitive impairment ($p=0.001$).
Jennings, 2004[213]	SLE	85	40.4	115 scans in 85 SLE patients in which 39 (34%) were normal, 70 (60%) had WMH, 50 (43%) had brain tissue loss, 24 (21%) had infarcts and 6 (5%) had haemorrhage.
Sanna, 2000[214]	SLE	68	38.0	Among 68 patients, 24 showed overt neuropsychiatric manifestations; none had acute presentation at time of scanning. Abnormal MRI was found in 30/68 (44%). Neuropsychiatric manifestations are significantly associated with serum antibody against anti-glial fibrillary acidic protein.
APS = antiphospholipid syndrome. FLAIR = fluid attenuated inversion recovery. MCA = middle cerebral artery. NPSLE = neuropsychiatric systemic lupus erythematosus. RA = rheumatoid arthritis. SLE = systemic lupus erythematosus. SS = Sjogren's syndrome. WMH = white matter hyperintensities				

Discussion

Brain damage from any type of stroke, from ischemic and hemorrhagic stroke, and 'silent' vascular damage such as WMHs is increased in most rheumatic diseases. Most data are for stroke in general, but ischemic and hemorrhagic stroke were also increased in our new pooled analyses of RA and SLE, as were silent vascular disease markers.

RA, SLE, AS, gout and to a lesser degree psoriasis carry a higher risk of stroke over the general population. Stroke incidence varies across rheumatic diseases (Table 4.4) and appear higher than the general population (for example Rothwell et al.[202] report annual incidence rates for ischaemic and haemorrhagic stroke as 141 (95% CI 127 to 156) and 12 (9 to 17) per 100,000 population, respectively).

Rheumatic disease patients aged under 50 have a particularly high stroke risk compared with the general population. There was no additional stroke risk in osteoarthritis. Other rheumatic diseases are understudied. While increased stroke risk may reflect impact on lifestyle through the physical effects of rheumatic diseases, the possibility that increased systemic inflammation affects the brain directly is suggested by the higher stroke risk in inflammatory versus non-inflammatory arthropathies. A better understanding of stroke in rheumatic disease would help focus clinical practice on prevention of vascular brain damage, including early lifestyle interventions and any vascular prevention role for anti-inflammatory agents, in these patients.

This is the first analysis to quantify stroke subtype rates and risk in rheumatic disease, including by age. Our results are comparable with Holmqvist et al's.[55] meta-analysis of 10 cohort studies of stroke and its subtypes in SLE, including that stroke risk is increased in the under 50's, but we expand the analysis by including other rheumatic

diseases. We were limited by the different methods used in the primary studies, although we attempted to correct for this by using random effects meta-analysis. Consistency of reporting of stroke rates is a recognised problem and future studies should attempt to standardise their methods[215].

The increase in risk of vascular disease in RA and SLE is perhaps expected, but we also note the almost doubling of stroke in gout (1.71, 1.68 to 1.75; n=9,951 strokes, n=202,033 patients) over the general population perhaps due to the relationship between gout and metabolic syndrome or to uric acid's independent association with ischaemic and haemorrhagic stroke subtypes[216].

The review's strengths include data from large population-based studies, although we only included English language studies. We could not adjust for vascular risk factors or treatments, limiting generalisability, and cannot exclude the possibility of study bias. Patients with rheumatic disease are often assiduously monitored (due to the disease and treatments) and so might have minor neurological problems investigated more compared to the general population.

Data on *ischaemic* stroke subtypes were limited to two studies in SLE[166,199]. Many more studies reported on SVD features among patients with several rheumatic diseases. While the small size and disparate reporting precluded meta-analysis, the general impression was of more vascular lesions in rheumatic diseases.

The increased stroke risk at earlier ages uses data from 340,548 patients (11,879 strokes). The excess risk was almost double that of the general population, highest in those <50 years, and declined steadily to approach that of the general population above age 65. However there was heterogeneity in study reporting and inconsistencies in age

categories (despite guidance to use mid-decade age bands[215]). The clear trend for higher stroke risk <50 years suggests that atherosclerosis is unlikely to be the sole pathogenic driver. Systemic inflammation may play a role. The increased risk at younger ages might reflect more rheumatic disease activity before the inflammation is well controlled.

The risk of any stroke, ischaemic or haemorrhagic stroke, and MRI findings seem worse in inflammatory arthropathies (RA, SLE, AS, gout, psoriatic arthritis) than non-inflammatory arthropathies (OA), although we acknowledge the inflammatory component to OA which limits interpretation of our results which dichotomises the arthropathies on the basis of inflammatory/non-inflammatory. Inflammation is a risk factor for stroke[217] and plasma markers of inflammation (CRP, TNF-a, IL-6) are associated with stroke[127] and increased WMH burden. Antiphospholipid (APL) antibodies increase the risk of thrombus: in rheumatic populations, strokes (and pre-clinical brain abnormalities such as WMH) are more common in those with APL than those without[204,207,208]. In those with stroke, APL are associated with stroke[218] and increase the stroke risk fivefold in those <50 years[219]. Conversely, Mikdashi et al.[199] found no association between baseline anti-cardiolipin antibodies and future stroke among 238 SLE patients followed for 8 years (although 44 (18%) developed ischaemic stroke, and the authors did not control for anticoagulant/antiplatelet use) while cholesterol and hypertension predicted stroke, indicating that traditional stroke risk factors should be managed rigorously. Petri et al.[220] found homocysteine, a marker of endothelial dysfunction, to be an independent risk factor for stroke in SLE. Therefore inflammation generated in the rheumatic diseases may be at least in part responsible for the marked stroke risk and overt brain lesions, and increased risk at younger ages.

A large well conducted brain imaging population study with detailed stroke phenotyping is needed to fully characterise stroke subtypes including SVD in rheumatic patients. A clear picture of increased stroke risk in younger patients is established indicating a need to a) unravel the extent to which inflammation, lifestyle, anti-rheumatic treatments and risk factors (traditional as well as new) contribute to stroke risk and b) whether aggressive management of these risk factors including inflammation, can ameliorate the stroke risk. Imaging features such as WMH might assist in identifying rheumatic disease patients who are at particularly high risk of stroke, as do WMH in the general ageing population[21].

Chapter 5: Cerebrovascular disease including stroke subtypes in patients with rheumatic diseases in NHS Lothian: a 15-year data linkage study

Introduction

Arthritis patients die prematurely from cardiovascular disease[156]. RA is an independent risk factor[154,155] in this regard, while Chapter 4 showed that SLE and other inflammatory arthropathies also increase stroke risk. Chapter 4 provided new data on the excess risk of stroke by stroke subtypes over the general population in RA (ischaemic, OR 1.64 (1.32 to 2.05); haemorrhagic, OR 1.68 (1.11 to 2.53)) and SLE (ischaemic, OR 2.11 (1.66 to 2.67); haemorrhagic, OR 1.82 (1.07 to 3.09)). Less is known about other arthropathies. Less is known about the relationship between arthropathies and *ischaemic* stroke subtypes such as lacunar stroke.

A proportion of excess stroke risk in arthritis could relate to higher systemic inflammatory activity. Different types of arthritis (eg, RA / SLE representing inflammatory arthritis versus osteoarthritis / osteoporosis representing non-inflammatory arthritis) could be studied to assess the contribution of inflammatory disease mechanisms between stroke subtypes. Inflammation plays a major role in all stages of atherosclerosis (and hence large vessel stroke)[43–45] and is seen pathologically in the intracranial arteriolar walls and perivascular tissue in small vessel disease (SVD) stroke[46]. However, while inflammation in atheroma might be a direct result of the atheromatous process, the origin of the inflammation in the brain microvessels in SVD, whether intrinsic or secondary to systemic inflammation, is unknown.

Most studies investigate single arthropathies. Only one prior study[178] has looked at stroke risk among a range of arthropathies concurrently in one study. No studies have grouped a range of arthropathies into inflammatory versus non-inflammatory subtypes to assess burden of stroke or stroke subtypes between these groups.

In Chapter 4 we saw that stroke risk (for any stroke) in rheumatic patients was greatest among younger people, particularly <50 years. No studies have grouped a range of arthropathies and compared stroke subtypes by age versus stroke subtypes in the general population. Identifying increased risk across the lifecourse is clinically important, might unveil mechanistic or lifestyle insights and has implications for stroke prevention. Early management of stroke risk factors is important to help avoid accumulating damage that might precipitate an acute cerebrovascular event.

We used comprehensive data from a large regional rheumatology service linked to Scottish national hospital records to: (1) assess the number of strokes, including subtypes, in relation to several common rheumatic diseases; (2) group the arthropathies into inflammatory and degenerative (non-inflammatory) categories to see if there is a higher burden of all stroke or of stroke subtypes among the inflammatory group; (3) investigate age at stroke to confirm if stroke in inflammatory versus degenerative arthropathies occurred at a younger age in this data and (4) set our findings in context by comparing to stroke incidence rates and age at stroke from the general population.

Methods

This is a cohort study, with ascertainment of rheumatic cases identified in an audit and extraction of data on antecedent vascular events in the preceding 15 years.

Prospective audit of rheumatology attendees

We undertook an audit of new referrals and follow-up patients attending outpatient clinics at the rheumatic diseases unit within NHS Lothian over a 24 month period from January 2011 to December 2012. Demographic details, clinical diagnoses, anti-rheumatic treatments and other relevant information were collected. A national data linkage search for cerebrovascular events was conducted for the patients in the audit.

National data linkage search

Every person in Scotland that is registered with a general practitioner (GP) or who has attended a hospital is given a unique reference number known as a Community Health Index (CHI) number. We sent the rheumatology patient CHI numbers to NHS Services Scotland to query its national hospital admissions database, SMR-01, to find episodes relating to cerebrovascular disease. The SMR-01 database records every general acute inpatient and day case admission in Scotland as part of the National Data Catalogue (http://www.ndc.scot.nhs.uk/Data-Dictionary/SMR-Datasets/). SMR-01 was upgraded in 1997 to cater for the introduction of version 10 of the International Classification of Diseases (ICD-10) classification system and so we searched over 15 years, from May 1997 to December 2012. We were interested in any of 11 codes (see below) of relevance to stroke. There is no ability to record ischaemic stroke subtypes in ICD-10.

ICD-10 codes

G45 – Transient ischaemic attack
I60 – Subarachnoid haemorrhage
I61 – Intracerebral haemorrhage
I62 – Other non-traumatic intracerebral haemorrhage (eg, subdural)
I63 – Cerebral infarction
I64 – Stroke, not specified as haemorrhage or infarction
I65/66 – Occlusion and stenosis, not resulting in cerebral infarction
I67 – Other cerebrovascular disease
I68 – Cerebrovascular disease classified elsewhere
I69 – Sequelae of cerebrovascular disease

Research ethics

The CHI numbers were removed after the database query so all analyses were on anonymised data. Both the NHS Lothian and NHS Services Scotland Caldicott Guardians approved the release of data under reference XRB13145. As such, research ethics committee approval was not required.

Grouped rheumatic categories

We grouped 18 rheumatic diagnoses into eight categories: ***RA*** (rheumatoid arthritis), ***seronegative*** (psoriatic arthritis, ankylosing spondylitis, reactive arthritis (Reiter's)), ***vasculitis*** (polymyalgia rheumatica and temporal arteritis, Churg Strauss vasculitis, polyarteritis nodosa, Takayasu disease), ***mixed*** (systemic lupus erythematosus, Sjogren's, scleroderma, mixed connective tissue disease), ***crystal*** (gout, pseudogout), ***osteoarthritis***, ***osteoporosis*** and ***other***. 'Other' was used when a diagnosis was provisional awaiting test results or was unclear.

We also grouped patients into inflammatory (all diagnoses except osteoarthritis and osteoporosis) and non-inflammatory (i.e., degenerative: osteoarthritis and osteoporosis) arthropathies. Some patients had a single rheumatic diagnosis of "Other" and therefore could not be dichotomised. Where a patient had multiple rheumatic diagnoses, we defined 'non-inflammatory' as requiring all diagnoses to be non-inflammatory.

Grouped stroke categories

'All strokes' were defined as any of: cerebral infarcts, intracerebral haemorrhage (ICH), subarachnoid haemorrhage, transient ischaemic attack (TIA), non-traumatic subdural bleeds or unspecified strokes. ICH, subarachnoid haemorrhage and non-traumatic subdural bleeds were grouped into one '*bleeds'* category for some analyses.

Comparison to population data and statistics

A patient rather than an event is the unit of analysis in this study. We calculated stroke incidence rates and placed stroke by subtype into 10-year age bands and compared these data with data from the general population[202,221]. We assessed differences in proportions by χ^2 test and differences in means for continuous data with Student's *t* test using the statistical programming language R version 3.0.1 (http://www.r-project.org/)[141]. We used logistic regression to adjust for age and sex when comparing cerebrovascular event versus no event among different groups. We considered a p value of <0.05 as significant.

Results

Audit and database query

The audit recorded 6,681 visits between January 2011 and December 2013, which we linked to national cerebrovascular events covering 15 years. We excluded repeat visits during the audit period (n=31 patients), visits from new patients that did not result in a rheumatic diagnosis (n=19) and coding errors (n=18) which left 6,613 patients available for analysis. The linkage query returned 37,619 records, being any hospitalisation for any purpose in our rheumatic patients and there were 347 cerebrovascular disease event episodes among 224/6,613 (3.4%) patients (66% women). Of 224 patients with a cerebrovascular event, 63 (28%) had multiple events and 53 (24%) had more than one rheumatic diagnosis (Figure 5.1)

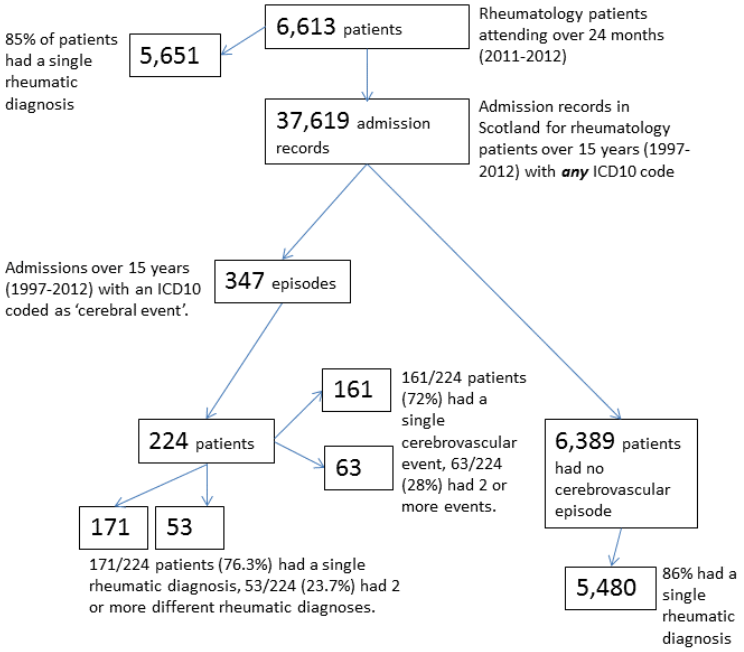


Figure 5.1 Schematic of audit data and national linkage search

There were 4,527/6,613 (68.5%) women in the audit. The mean age of those attending clinic was 56 years (range 14–95 years). The mean age at cerebrovascular event was 63.7 ± 12.9 years (Table 5.1).

Table 5.1 Overview cerebrovascular events among 224 rheumatology patients

	All	**Women**	**Men**
Number of persons (%)	**6,613**	4,527 (68.5%)	2,086 (31.5%)
Age at clinic visit (years)	**56.0**	56.5	55.0
Number having an event (%)	**224**	149 (66.5%)	75 (33.5%)
Proportion*	**3.4%**	3.3%	3.6%
Age at event (years)	**63.7**	63.7	63.6
* Number of rheumatology patients with an Event as a proportion of all rheumatology patients			

Inflammatory versus non-inflammatory

We classified 4,088/6,613 (62%) rheumatology patients as inflammatory and 664/6,613 (10%) as non-inflammatory. Of these, 157/4,088 (3.8%) and 28/664 (4.2%) had cerebrovascular events (Table 5.2).

Table 5.2 Cerebrovascular events among rheumatology patients split by inflammatory and non-inflammatory arthritis (excludes 1,861 "Others")

	Inflammatory	**Non-inflammatory**	**P value**	**Others**
Patients with event	157	28	0.72	39
Patients with no event	3,931	636	---	1,822
Total	**4,088**	**664**	**---**	**1,861**
Proportion of patients with event	3.8%	4.2%	---	
Mean age at event (years)	64.0	61.5	0.23	
Mean age at clinic visit (years)	68.2	66.9	0.51	
Others could not be dichotomised inflammatory/non-inflammatory				

Almost a third (1,861/6,613 (28%)) of patients had a single rheumatic diagnosis of "Other" and could not be classified as inflammatory / non-inflammatory, including 39

patients with cerebrovascular events. There were no significant differences in age at event (p=0.23) or the proportion (p=0.72) of rheumatology patients having a cerebrovascular event between arthropathies grouped as inflammatory (n=4,088) versus non-inflammatory (n=664) (Table 5.2).

Inflammatory arthritis was not associated with cerebrovascular events before and after (Table 5.3) adjusting for age and sex. Age was predictive of events: a year's increase in age resulted in a 6% increase in likelihood of having an event (Table 5.3).

Table 5.3 Logistic regression showing odds ratios for three parameters in estimating cerebrovascular event versus no event

	OR	**95% CI**
Inflammatory arthritis	0.93	0.62 to 1.44
Age (year)	1.06 *	1.05 to 1.08
Male sex	1.28	0.93 to 1.75
CI = confidence interval, OR = odds ratio. Compares odds of having a cerebrovascular event versus no event in n=4,088 inflammatory and n=664 non-inflammatory patients. * p<0.0001.		

RA versus osteoarthritis

We repeated the analysis comparing only patients with a single diagnosis of either RA (n=1,768) or osteoarthritis (n=583) and found no statistical difference in age between the groups, no difference in the proportion of any cerebrovascular event, and no difference in any ischaemic or haemorrhagic stroke versus no event.

Grouped rheumatic categories

The proportion of cerebrovascular events by grouped rheumatic categories were: RA 3.8%, crystal 4.4%, seronegative 2.0%, vasculitis 6.3%, mixed 1.7%, osteoarthritis 3.6% and osteoporosis 9.0% (Table 5.4).

Table 5.4 Cerebrovascular events among rheumatology patients by grouped category

	No event	Event	Event as %
Inflammatory			
RA	1,700	68	3.8%
Crystal	109	5	4.4%
Seronegative	751	15	2.0%
Vasculitis	193	13	6.3%
Mixed	293	5	1.7%
Sub-total	**3,046**	**106**	**3.4%**
Non-inflammatory			
Osteoarthritis	562	21	3.6%
Osteoporosis	50	5	9.0%
Sub-total	**612**	**26**	**4.1%**
Other	1,822	39	2.1%
Total	**5,480**	**171**	**3.0%**

Stroke subtypes

There were 151 patients with some form of stroke: 54 infarcts, 30 TIAs, 7 ICHs, 14 subarachnoid haemorrhages, 5 non-traumatic subdural bleeds and 41 unspecified strokes (Table 5.5). There were no statistically significant differences in the numbers of infarcts or bleeds among inflammatory versus non-inflammatory arthropathies as a proportion of all strokes (p=1.0) nor as a function of the entire cohort (p=0.79). Grouping unspecified strokes with ischaemic strokes did not alter these results.

Table 5.5 All stroke and stroke subtypes

	Total	Inflammatory	Non-inflammatory	Other*
Ischaemic				
Infarcts	54	37	6	11
TIA	30	21	3	6
Unspecified	41	32	4	5
Sub-total	**125**	**90**	**13**	**22**
Haemorrhagic				
ICH	7	5	1	1
Subarachnoid	14	11	1	2
Subdural	5	3	1	1
Sub-total	**26**	**19**	**3**	**4**
Any stroke	**151**	**109**	**16**	**26**

Numbers are patients. * Other = Unable to assign to inflammatory/non-inflammatory. ICH = intracerebral haemorrhage. TIA = transient ischaemic attack.

General population comparison of stroke incidence rates and age at stroke

The number of any strokes equated to 152 strokes per 100,000 person-years. Lee et al.[221] and Rothwell et al.[202] report stroke incidence in the UK as ranging from 104–227 per 100,000 person-years. The mean age at event in our data was 63.7 ± 12.9 years with events peaking in the 55–64 years age band (Table 5.6 and Figure 5.2).

Table 5.6 Stroke subtypes by 10-year age bands across all rheumatic diagnoses

	25 to 34	35 to 44	45 to 54	55 to 64	65 to 74	75 to 84	85+
Ischaemic							
Infarcts		3	8	15	13	15	
TIA		4	3	13	6	3	1
Unspecified	2	2	5	13	9	8	2
Sub-total	**2**	**9**	**16**	**41**	**28**	**26**	**3**
Haemorrhagic							
ICH		1		4	2		
Subarachnoid		6	3	2	2	1	
Subdural			2	1	1	1	
Sub-total		**6**	**5**	**7**	**5**	**2**	
Total	**2**	**15**	**21**	**48**	**33**	**28**	**4**

Numbers are patients. ICH = intracerebral haemorrhage, TIA = transient ischaemic attack.

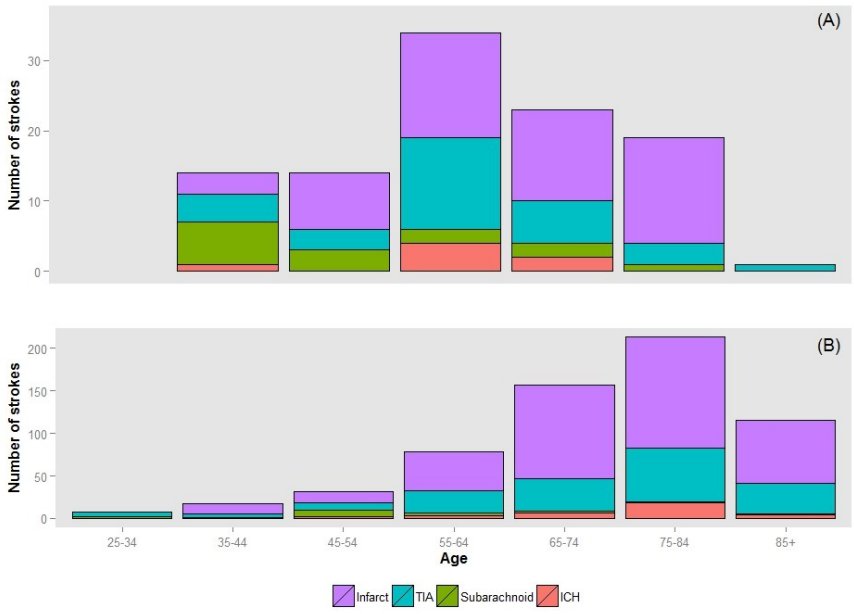


Figure 5.2 Stroke subtypes by 10-year age bands. Panel (A) among rheumatic patients in south-east Scotland; panel (B) among the general population[202]

Discussion

This was a large national retrospective data linkage study involving 6,613 patients with various rheumatic diagnoses attending a regional specialist rheumatology clinic. We found 224 cerebrovascular events (3.4%).

Although our stroke incidence rate is within the range expected from the general population, the strokes occurred on average two decades earlier than in the general population. There was no difference in the proportion of strokes occurring between patients classified as inflammatory versus non-inflammatory, although a third of the data could not be used as the diagnoses were incomplete.

We classified a patient as 'non-inflammatory' only if there was a diagnosis of osteoarthritis, osteoporosis or both in patients with two diagnoses but verified our results by repeating the analysis by comparing only patients with a single diagnosis of either RA or osteoarthritis. Moreover, there was no association of any event with RA after controlling for age and sex. RA is generally considered a more aggressive disease requiring assiduous monitoring than osteoarthritis, with RA patients less likely to miss a clinic visit. Meanwhile osteoarthritis is a very common disorder so perhaps only the more severe osteoarthritis referrals and follow-ups may be represented in our data. Furthermore increasing the non-inflammatory denominator could lead to detection of a difference in events between RA and osteoarthritis, although it would take a roughly doubling of the non-inflammatory denominator in our data to show any difference.

We captured a range of strokes. However, mild strokes and TIAs are generally not admitted to hospital in NHS Lothian but instead followed-up as outpatients in specialist neurovascular clinics and consequently are not captured in the national

database which requires an admission or in-patient day case visit. Hence TIA and mild strokes are likely to be under-represented in this analysis whereas haemorrhagic stroke which is more often severe is probably more completely captured. It was not possible to link to data on stroke outpatient records.

We did not review individual clinical records nor the results from imaging and so are unable to verify if the cerebrovascular events were coded correctly in the national database, however studies have validated the use of administrative data in stroke research[222]. We are unable to comment on modifiable risk factors such as smoking, diabetes, hypertension, relative lipid levels, high homocysteine, sedentary lifestyles, etc. A proportion of stroke risk in rheumatic patients may be a function of the inevitable lifestyle restrictions: joint pain, swelling, lack of motivation and depression limit activity and exercise, potentially increasing stroke risk through weight gain, higher blood pressures, etc.

We included osteoporosis with osteoarthritis when creating a non-inflammatory group since patients with osteoporosis are followed-up by rheumatology, although we note this is primarily a metabolic bone disease.

We did not have date of rheumatic diagnosis nor date of commencement of treatments and could not analyse duration of rheumatology disease and cerebrovascular events. The retrospective design is limiting; this was not an inception cohort from 1997 and thus those with rheumatic diseases that died from stroke between 1997 and 2011 will not have been captured in the data.

Our incidence rates are crude (the nature of this linkage study does not allow for true "observed years at risk" to be known) and comparison with general population data is

merely illustrative. Our overall rates are slightly higher than Lee et al.[221] but lower than Rothwell et al.[202], possibly as the number of minor strokes we found from the linkage data will be under-stated.

Not all prior studies have associated rheumatic disease with an increased risk of stroke. In meta-analysis, Levy et al.[223] found no additional risk of stroke (n=79 any type strokes) in 2,235 RA patients versus the general population (OR 1.14, 95% CI 0.86 to 1.51), although a larger more recent meta-analysis did find an association (pooled stroke incident rate ratio 1.91 (95% CI 1.73 to 2.12))[170]. In Chapter 4 it was shown that the increased risk of ischaemic and haemorrhagic stroke in RA was 1.64 (1.32 to 2.05) and 1.68 (1.11 to 2.53) respectively.

Stroke occurred early: our data were clustered around the 55–64 years age band, two decades younger than the average age of stroke in the general population[202]. Other studies[161,164,166,178,189] have reported a trend towards increased stroke risk earlier in life among rheumatic patients, including our own findings (Chapter 4). Ours is the first study to group several different rheumatic diseases to compare age of stroke by subtype versus stroke subtypes in the general population.

The persistent, non-resolving inflammatory mechanisms present in many rheumatic diseases could accelerate stroke processes resulting in stroke earlier in life (perhaps before the inflammation is brought under control), although we found no difference in the proportion of strokes between arthropathies grouped as inflammatory versus non-inflammatory, nor between RA and osteoarthritis when analysed separately – although the non-inflammatory patients captured here may be more severe than non-inflammatory arthritis patients in general and we lost power through not including

many patients classified as 'Other'. Those having a cerebrovascular event classified as inflammatory were slightly (non-significantly) older than those classified as non-inflammatory (64 *v* 61.5 years). The use of logistic regression to adjust for age and sex did not alter our findings. Either a larger sample is needed to detect a difference, or non-inflammatory arthropathies, particularly those severe enough to be referred to a specialised rheumatology service, are equally subject to more stroke risk. Moreover, uncontrolled osteoarthritic joint pain might itself trigger an inflammatory cascade with systemic consequences, including effects on the brain.

Implications and future work

Rheumatologists should consider the possibility of cerebral involvement in all rheumatology patients (not solely those with presumed vulnerability such as RA, SLE and vasculitis patients), and note that stroke is more likely early in life compared to stroke in the general population. Assiduous risk factor management to help reduce the risk of stroke is essential and could be beneficial if started early. Rheumatology patients are often encouraged to engage in exercise as it is helps their rheumatic condition, however doing so will also reduce stroke risk. Brain imaging studies involving rheumatic patients are likely to be helpful in understanding the brain's involvement in, and response to, rheumatic diseases. Large, prospective, longitudinal studies that follow the brain in patients starting different forms of anti-inflammatory treatments would be useful. More studies involving the lesser studied rheumatic diseases, including the non-inflammatory arthropathies, are also needed.

Chapter 6: A pilot MRI neuroimaging study of patients diagnosed with systemic lupus erythematosus ("the SLE study"): background, rationale and methods

Introduction

SLE is a chronic inflammatory autoimmune disease, mostly affecting women, which is associated with an increased risk of stroke[55,56] for reasons which are incompletely understood (reviewed in Chapter 4). SLE patients also complain of fatigue, 'brain fog', cognitive impairment and other neurological involvement[224]. At least three prospective studies (n=1,385) document rates of at least one episode of psychiatric disturbance over several years of around 40%, as well as headaches, cognitive disturbance and neurological symptoms that fall short of stroke[211,225,226]. These symptoms are disabling, unpleasant and contribute substantially to the burden of disease.

Fatigue[60] is a common but unexplained feature of SLE which increases distress and lost work days. Fatigue in SLE has been associated with an increased burden of brain WMH in one study[61], suggestive of a biological basis for fatigue. Cognitive decline in SLE patients has also been reported[59]. Cognitive decline also occurs with WMH in SVD, therefore SVD might explain both cognitive decline and fatigue in SLE, possibly linked through inflammation.

Cerebral small vessel disease

SVD is an intrinsic disorder of the brain's perforating arterioles[18]. Features range from asymptomatic WMH and other brain imaging biomarkers[19] of SVD to symptomatic lacunar stroke, which accounts for a quarter of all ischaemic strokes. SVD is a major

cause of cognitive impairment and dementia, and also results in depression and gait and balance problems[18].

In sporadic SVD, inflammation and cell infiltrates are seen in the perforating arteriolar walls and microglial activation is seen in the perivascular tissue on pathology[46,47]. The source of the inflammation is not known, whether intrinsic or systemic, but is associated with raised plasma markers of inflammation in healthy older subjects[48]. C-reactive protein (CRP), one of the main clinical measures of inflammation, was associated with lacunar infarcts in a recent large (n=519) study, independent of age and vascular risk factors[49]. Factors that contribute to damage of the endothelial lining of brain vasculature such as immune complex formation and complement activation/deposition and occur in SLE, might trigger cerebrovascular inflammation in SLE. We hypothesized that one explanation for increased stroke risk in patients with SLE could be via the effects of systemic inflammation on cerebral small vessel integrity.

White matter microstructural damage

As brain white matter is disrupted in SVD we also hypothesized that patients with SLE, but no overt neurological dysfunction, would show subclinical white matter damage. Diffusion tensor magnetic resonance imaging (DT-MRI) and quantitative tractography are techniques that can map the brain's white matter tracts (bundles of individual white matter fibres known as axons) and provide biomarkers for microstructural integrity. Water molecules preferentially diffuse along the principal fibre direction in healthy white matter, while loss of structural integrity, which has a deleterious effect on brain function, increases free water movement. The magnitude

and directionality of water molecule diffusion can be quantified within segmented tracts using biomarkers such as mean diffusivity (MD) and fractional anisotropy (FA),[227] with low MD and high FA indicating structurally intact white matter. A study in a large sample (n=420) of older people in their early seventies showed that white matter tract integrity measured using quantitative tractography is a global property of the brain[227]. That is, lower FA in one tract tends to be associated with lower FA in all other tracts, and the first unrotated principal component (considered to be a general factor of white matter integrity) explained 38% of the variance across white matter tracts. Additionally, in that study, the general factor of white matter integrity was related to general cognitive ability.

Nine small (average n=24 patients) studies[57,228–235] showed microstructural white matter tract damage in SLE and neuropsychiatric SLE (NPSLE) versus healthy controls. However, none of these studies in SLE investigated whether subvisible DT-MRI-detected brain damage associates with fatigue or cognitive function.

Hypothesis

We hypothesized that patients diagnosed with SLE have brain MRI evidence of SVD, and this could be the biological basis for fatigue and cognitive decline, and might explain increased stroke risk.

Pilot study

This cross-sectional brain MRI study ("the SLE study") was a pilot study designed to test our hypothesis, establish and test working practices and protocols (e.g., to see if the SLE patients would tolerate the MRI exam, and to see if our techniques for image

analysis would work well in this cohort) and to generate data which could be used to inform and power a future multi-centre study. A grant application to fund the study was developed (by the author of this thesis, supported by his supervisor) and submitted to the charity Lupus UK and granted in November 2013 (award letter in Appendix B).

This pilot study is part of work to improve understanding of how inflammation affects the brain vessels and tissue and relates to symptoms, with the aim ultimately of improving prevention and treatment. The detection of early changes in the brain could influence the management of patients with SLE to prevent disabling neurological symptoms and progressive brain damage. This knowledge will also contribute to understanding of how inflammation affects the brain to improve prevention and treatment of SVD-related lacunar stroke, cognitive decline and dementia.

Aims

In addition to piloting this brain study in SLE patients, our aims were to:

[these results are presented in Chapter 7] (1) measure and compare imaging biomarkers of SVD in patients with SLE with sex- and age-matched healthy controls and patients with mild stroke including small vessel type; (2) compute a total burden of SVD score and determine associations with vascular risk factors, plasma biomarkers of inflammation, endothelial dysfunction and coagulability, and SLE disease activity and damage;

and:

[these results are presented in Chapter 8] (3) compare water diffusion biomarker values measured in a number of major white matter tracts using quantitative

tractography with sex- and age-matched healthy controls; (4) assess associations between MD and FA and other variables as well as the relationship with age; (5) identify if fatigue or cognitive function in SLE were related to DT-MRI parameters, SLE disease activity or plasma biomarkers of inflammation, endothelial dysfunction and coagulability and; (6) consider the totality of the data on DTI-MRI in SLE and NPSLE by meta-analysing the current and all prior studies.

Methods

SLE patients

We prospectively recruited patients with SLE – including patients already recruited to the Scottish Lupus Exchange database (SLEx) (UK Clinical Trials ID 15489) – who attended a specialist SLE clinic in NHS Lothian between April and December 2014. The SLEx is new national network of rheumatologists and a patient registry project formed in 2008 and funded initially to 2018 that longitudinally monitors SLE disease activity, progression and treatments. The SLEx is co-ordinated from Dundee University and includes participating centres in Fife, Grampian, Greater Glasgow and Clyde, Highland, Lanarkshire, Lothian and Tayside. The mission for SLEx is stated as:

> 'SLEx aims to raise awareness and understanding of SLE and other related connective tissue diseases and to promote high standard clinical care and scientific research of these conditions in Scotland and further afield'.

All patients were seen by a consultant rheumatologist. SLE was diagnosed according to updated American College of Rheumatology 1997 criteria[236]. A person is said to have SLE if they meet four from 11 of the following clinical criteria: *malar rash*; *discoid rash*; *photosensitivity*; *oral ulcers*; *arthritis*; *serositis*; *renal disorder*; *neurologic disorder*; *haematologic disorder*; *immunologic disorder*; *antinuclear antibodies* (further details are presented in Appendix C that draws from the earlier 1982 guidelines[237], which remain essentially unchanged in the 1997 update other than a revision to criterion 10 "immunologic disorder").

Regulatory approvals and informed consent

The author of this thesis completed the ethical approval process using the Integrated Research Application System (https://www.myresearchproject.org.uk/) and the project received research ethics committee (REC) approval (South-East Scotland REC 01, 14/SS/0003; approval letter in Appendix D) and local R&D approval (NHS Lothian; approval letter in Appendix E). A protocol was developed (by the author of this thesis, supported by his supervisor; Appendix F) and all participants were sent an information sheet about the study (Appendix G). Written informed consent (Appendix H) was obtained from all participants.

Inclusion and exclusion criteria

We included adult (aged 18+ years) patients with a diagnosis of SLE who were able to self-consent. We excluded those unable to undergo MRI (e.g., patients fitted with a cardiac pacemaker) and those with prior significant head injury or known neurological disease such as multiple sclerosis. We avoided scanning during concurrent acute illness like infection to avoid the acute phase immune response which would influence inflammatory markers.

Overview of data collection for SLE patients

We developed a data collection form (Appendix I) for demographic and other variables, measured SVD and DT-MRI imaging biomarkers (detailed below) and used validated tools (detailed below) to assess fatigue, anxiety, depression, current cognitive function and premorbid intelligence. The tests were chosen for their ease of use, practicality, validity and relevance to medical care and patients. Therefore the

cognitive tools did not test every aspect of cognitive function and intelligence in minute detail but focussed on collecting information in major domains in ~20 mins. We measured plasma markers of inflammatory activity and endothelial dysfunction, SLE disease activity and accumulated systemic damage (detailed below). An overview of the data collected is presented in Figure 6.1.

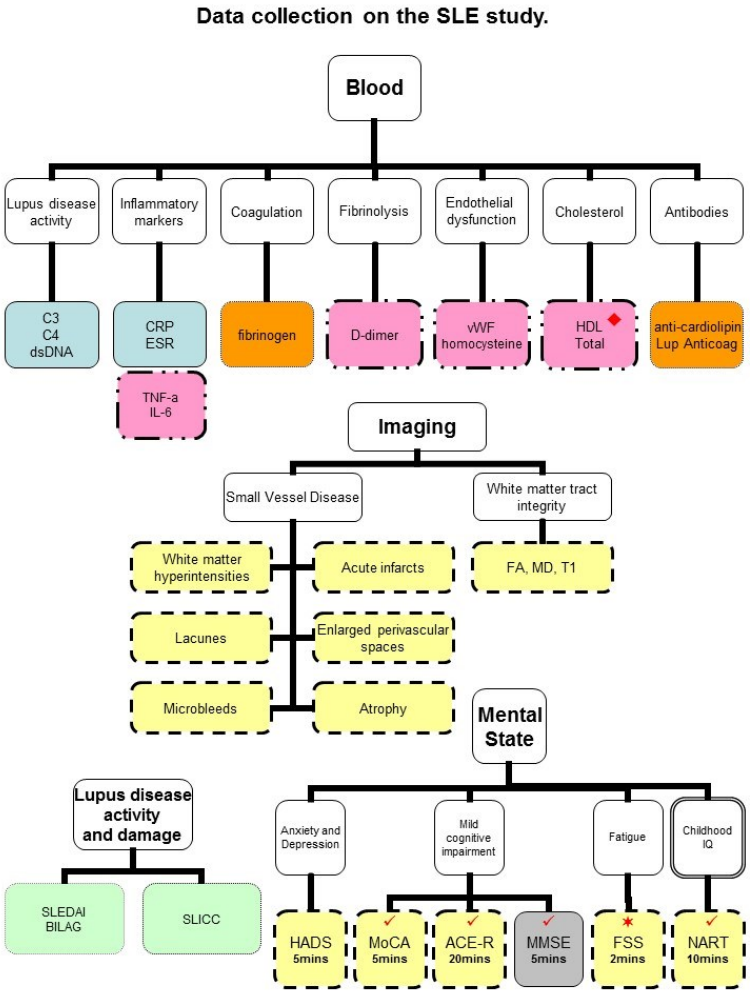


Figure 6.1 Data collection on the SLE study.

ACER = Addenbrooke's cognitive examination – revised, BILAG = British Isles lupus assessment group 2004, CRP = C reactive protein, dsDNA = double stranded DNA, ESR = erythrocyte sedimentation rate, FA = fractional anisotropy, FSS = fatigue severity scale, HADS = hospital anxiety and depression scale, HDL = high density lipoprotein, IL-6 = interleukin-6, MD = mean diffusivity, MoCA = Montreal cognitive assessment, MMSE = mini mental state examination, NART = national adult reading test, SLEDAI = sytemic lupus erythematosus disease activity index, SLICC = systemic lupus international collaborating clinics, TNF-a = tumour necrosis factor alpha, vWF = von Willebrand factor.

Healthy controls

We obtained control data from healthy volunteers aged between 25 and 65 years, recruited by poster campaign at the same health region. Healthy volunteer subjects were recruited if they were native English speakers, were not on any long term medication, had not been diagnosed with diabetes or hypertension, had not undergone previous cranial surgery, had alcohol consumption levels within the UK national safety guidelines and were able to undergo brain MRI. The study was funded by the National Institute for Health (grant R01 EB004155-03), approved by the Lothian Research Ethics Committees REC (05/S1104/45) and subjects gave written informed consent.

Stroke controls

We also compared the SLE patients with patients with first-ever mild (non-disabling) stroke including of small vessel (lacunar) type, recruited from the same health region via the regional stroke service. The study, known as Mild Stroke Study 2 (MSS 2), was funded by the Wellcome Trust (grant WT088134/Z/09/A), approved by the South-East Scotland REC 01 (09/S1101/54) and subjects gave written informed consent. A stroke specialist determined the ischaemic stroke subtype (lacunar or mild cortical) using the Oxfordshire Community Stroke Project (OCSP)[15] clinical classification and confirmed by imaging. Similar tests were used for assessing premorbid cognition and mood.

Vascular risk factors – SLE patients

Medical histories including cardiovascular risk factors such as smoking status, prior cerebrovascular events, hypertension and diabetes were recorded in SLE patients.

Height and weight were measured and body mass index (BMI) calculated using the NHS online tool (http://nhs.uk/Tools/Pages/Healthyweightcalculator.aspx). We measured blood pressure three times using an Invivo Research Inc 3150 MRI-compatible patient monitor (before the MRI scan, after the scan and at the end of the study visit) to derive an average blood pressure reading, and we also identified patients on hypertension medication from medical notes. We dichotomised patients as hypertensive or not, and also classified them with the British Hypertension Society 6-point scale, from optimal to severe[238].

SLE activity and damage scores – overview

SLE disease activity was assessed by an experienced rheumatology nurse specialising in SLE who interviewed each patient and had access to all medical data and blood results using the Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K)[239] and British Isles Lupus Assessment Group 2004 (BILAG)[240] tools. Accumulated permanent damage was assessed with the Systemic Lupus International Collaborating Clinics (SLICC)[241,242] tool.

SLE activity and damage scores – further details

The SLEDAI-2K (Appendix J) is a 24-item checklist of important factors in disease activity from which the assessor decides whether each manifestation is present or absent in the preceding 10 days[243]. The scores are weighted such that more serious manifestations like seizures and other central nervous system disorders are given more prominence in the final score. Higher scores reflect more current disease activity. The

maximum theoretical score is 105 but in practice patients rarely score more than 45 and scores higher than eight are considered active disease[244].

The BILAG (Appendix K) consists of 97 questions (known as "items") over nine systems (known as "categories": constitutional, mucocutaneous, central nervous system, musculoskeletal, cardiovascular/respiratory, abdominal, renal, ophthalmic and haematological) and is based on "intention to treat"[240,245]. The answers to the questions are entered into the web-based i-BLIPS (British Lupus Integrated Prospective System) system and include responses on Likert scales, Yes/No answers and the input of serological values. The i-BLIPS software determines a "score" that summarises the involvement of each of the nine category systems as follows: A (Action), B (Beware), C (Content), D (Once involved but no longer active) and E (Never involved). The most active score in each system, grade A, is defined as "the individual clinical features, or combination of features, which would lead to the prescription of medium/large doses of corticosteroids (>20mg prednisolone or equivalent) and/or starting or increasing immunosuppressive drugs or high-dose anticoagulation"[240]. Scores are not intended to be summed into a global measure, although Yee et al.[246] showed the validity of a numeric conversion of the scores as follows: A=12, B=8, C=1, D=0 and E=0. We created numerical values from the nine individual system scores following the approach of Yee et al.[246] and then summed these to create a global BILAG disease activity score per patient.

The damage index SLICC (Appendix L) was used to assess permanent damage. SLICC measures accumulated damage across 12 organ systems arising since the onset of SLE where damage is defined as "irreversible change in an organ or system that has been present for at least six months"[247]. Use of the six month time frame allows for

permanent damage to be distinguished from active inflammation. Damage may be the consequence of active SLE disease, therapy, comorbid disease or any combination of these[247]. Higher scores reflect more accumulated damage.

Fatigue

The Fatigue Severity Scale (FSS)[58] was used to assess fatigue (Appendix M). The FSS asks nine questions with responses scored on a 7-point Likert scale (1 strongly disagree to 7 strongly agree). Higher scores indicate more severe fatigue. The mean (± SD) from normal healthy adults in the standardisation sample was 2.3 (± 0.7)[58]. In the present study, participants were asked to assess how they felt within the prior week.

Cognitive assessments

The Hospital Anxiety and Depression Scale (HADS)[248] was used to assess levels of anxiety and depression (Appendix N). The HADS is a short (about 2 minutes to complete) self-administered validated[249] questionnaire, designed to assess anxiety and depression. It asks 14 questions; the scores allow a patient to be categorised as 'case', 'borderline case' and 'non-case' for each of anxiety and depression. We received permission from the publishers of HADS, GL Assessment Ltd, to use this tool. We used this in preference to the Beck's Depression Inventory (used in MSS 2) because it can be completed independently.

Three validated screening tools were used to assess cognitive function: the Montreal Cognitive Assessment (MoCA)[250], Addenbrooke's Cognitive Examination – Revised (ACER)[251] and Mini Mental State Examination (MMSE)[252] (Appendices O, P and Q),

while the National Adult Reading Test (NART)[253] was used to adjust for premorbid intelligence (Appendix R). Further details follow.

The MoCA was designed as a rapid screening tool for mild cognitive impairments. It assesses different domains of cognition (e.g. attention, executive function, orientation, etc.) and is scored out of a maximum of 30 points. A score of 26 and above is considered normal. Copyright permission is not required.

The ACER is a brief cognitive test that assess five domains (attention/orientation, memory, verbal fluency, language and visuospatial abilities). The total score is 100 and higher scores indicate better cognitive functioning with scores below 88 leading to a suspicion of dementia. Administration takes about 15 minutes. Copyright permission is not required.

The MMSE tests a number of mental abilities, including memory, attention and language. The maximum score is 30 and scores of 27 or above are considered normal while scores of between 10 and 26 warrant further investigation for mild-to-moderate Alzheimer's disease, and below 10 indicates severe Alzheimer's. We received permission from the publishers of MMSE, PAR Inc, to use this tool.

The NART is a validated[254] estimate of childhood peak premorbid intelligence as it appears broadly resilient to age-related cognitive decline. The NART assesses ability to read aloud 50 irregular words that do not follow common grapheme-phoneme and stress rules[254]; the words can only be pronounced correctly if the person has a prior knowledge of the word. The maximum score is 50 correctly pronounced words. Reading ability is used as it is highly correlated with general IQ in the normal population. By adding NART as a term in the linear models (see **Statistical analysis**

below), we can adjust for premorbid intelligence, allowing us to assess changes in cognitive function from an inferred peak.

MRI

All subjects were scanned at 1.5T on a research-dedicated MRI scanner with an 8-channel phased-array head coil (GE, Milwaukee, WI). The scan protocol included an axial T2, axial gradient-recalled echo (GRE), axial fluid-attenuated inversion recovery (FLAIR), sagittal T2, high-resolution coronal 3D T1 volume, and whole brain DT-MRI sequences. The DT-MRI sequence consisted of three T2-weighted and 32 diffusion-weighted ($b = 1000$ s mm $^{-2}$) axial single-shot spin-echo echo-planar imaging volumes (field of view 240 × 240 mm, matrix 128 × 128, TR 13.75 s, TE 78.4 ms). Each volume comprised 56 contiguous 2.5 mm thick axial slices with 1.875 mm in-plane resolution. The scan protocol took ~50 mins. Detailed scanning parameters are presented in Table 6.1.

Table 6.1 Scanning parameters for the SLE study

SEQUENCE	Loc	FLAIR	T2	GRE	Sag T2 Cube	3D IR PREP	DTI (32 directions)	FSPGR 2	FSPGR 12
ORIENTATION		AX	AX	AX	SAG	COR	AX	AX	AX
TE		140	102	14		MIN FULL	MIN	MIN FULL	MIN FULL
TR		9400	8750	1420	3000		13750		
TI/prep time		2350				500			
FOV		24	24	24	24	24	24	24	24
SLICE THICK.		5	2.5	2.5	1	1.3	2.5	2.5	2.5
SLICE GAP		0	0	0	0	0	0	0	0
Acq. MATRIX		384 x 256	384 x 384	384 x 256	320 x 320	192 x 192	96 x 96	128 x 128	128 x 128
Padded (R x C)		512 x 512	512 x 512	512 x 512	512 x 512	256 x 256	128 x 128	256 x 256	256 x 256
Pixel width		0.47	0.47	0.47	0.47	0.94	1.875	0.94	0.94
Pixel height		0.47	0.47	0.47	0.47	0.94	1.875	0.94	0.94
Voxel depth		5	2.5	2.5	1	1.3	2.5	2.5	2.5
Resolution		2.133 pix/mm	2.133 pix/mm	2.133 pix/mm	2.133 pix/mm	1.067 pix/mm	0.533 pix/mm	1.067 pix/mm	1.067 pix/mm
FREQ. DIR		AP	AP	AP		SI	RL	AP	AP
BAND WIDTH		15.63	20.83	12.50		15.63		27.78	27.78
NEX		1	1	1	1	1	1	1	1
FLIP ANGLE				20		8		2	12
NO. SLICES		28	56	56	1 SLAB (180 locs)	1 SLAB (160 locs)	56	1 SLAB (62 locs)	1 SLAB (62 locs)
TIME of ACQ.	0:10	6:16	5:59	6:09	5:13	8:12	8:15	00:49	00:49

Image review and visual rating of the MRI scans

All MRI scans were reviewed by a consultant neuroradiologist blind to all other data. Imaging features of SVD were defined per STRIVE guidelines[19].

We defined WMH as punctate or diffuse areas in the white matter and deep grey matter of the cerebral hemispheres or in the brainstem that were 3 mm or larger in diameter, and hyperintense with respect to normal-appearing white and grey matter on T2 and FLAIR images. Deep and periventricular WMHs were coded 0 to 3 using the Fazekas[137] scale and summed to give a total WMH score (0 to 6) per subject.

Visible (enlarged) perivascular spaces (PVS) are seen as round (<3mm) or linear depending on the scan plane in relation to the orientation of the vessel[255] and their intensity is that of cerebrospinal fluid on T2-weighted MRI. They were assessed in the basal ganglia and centrum semiovale and scored as 0 (none), 1 (1–10 PVS), 2 (11–20), 3 (21–40) and 4 (>40) using a validated scale[138,255].

Lacunes[256] were defined as deep infarcts – distinguished from PVS due to their larger size (3–20 mm) – and their presence, including location in the brain, was noted. We used the GRE scans and the simplified Brain Observer Microbleeds Scale[139] to count microbleeds.

Cerebral atrophy was defined as enlargement of the ventricles (deep atrophy) and enlargement of the sulci (superficial atrophy) and scored accordingly by classifying each participant on a validated 6-point scale[257] against a template of normal reference brains. Figure 6.2 (overleaf) summarises the MRI features of SVD.

Total SVD score

A total SVD score (range 0–4)[258,259] was calculated from individual imaging features by awarding points as follows: 1 for any lacunes, 1 for any microbleeds, 1 for moderate to severe PVS in the basal ganglia (grade 2–4) and 1 for WMHs (deep tissue: Fazekas score 2 or 3 and/or periventricular: Fazekas score 3). The total SVD score correlated with both WMH volume (see below) (r = 0.61, p<0.0001) and summed total Fazekas score (r = 0.65, p<0.0001).

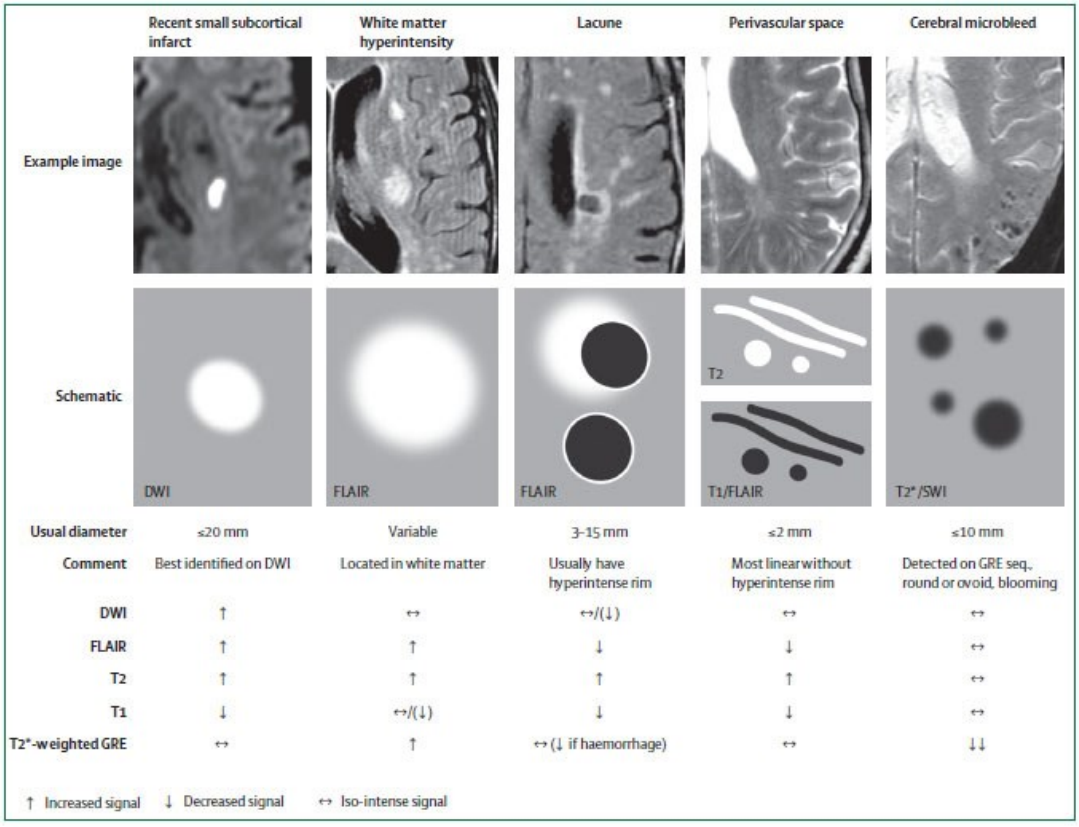


Figure 6.2 MRI findings for lesions related to SVD. Shows examples (upper row) and schemaic representation (middle row) of MRI features related to SVD, with a summary of imaging characteristics (lower row) for individidual lesions. DWI = diffusion-weighted imaging, FLAIR = fluid-attenuated inversion recovery, GRE = gradient-recalled echo, SWI = susceptability-weighted imaging. Reproduced from Wardlaw et al. *Lancet Neurol* 2013;12:822–38 with permission from the publishers Elsevier Ltd (license number 3832420235618 dated 19 March 2016).

Quantitative analysis of the MRI scans

All pre-processing and image analyses of the structural images was performed by the author of this thesis. All steps involved in generating the tractography data were performed by Dr Mark Bastin, Brain Research Imaging Centre.

Pre-processing

All images were converted from the Digital Imaging and Communications in Medicine (DICOM) international standard file format for medical images (http://dicom.nema.org) to the Neuroimaging Informatics Technology Initiative (NIfTI-1) format (http://nifti.nimh.nih.gov/nifti-1) using MRICRON (https://www.nitrc.org/projects/mricron). The axial T2-weighted scan was chosen as the target scan for registering the other scans to as it has the best resolution and so is the best representation of brain anatomy. Each subject's FLAIR, GRE and 3D T1 coronal scans were registered to the T2 using FLIRT[260] in FSL (http://www.fmrib.ox.ac.uk/fsl). Registered scans were manually inspected for accuracy, e.g., to ensure the scan orientation followed standard radiological convention (and were not 'flipped' during pre-processing).

Volumetric imaging measures – ICV, CSF, BTV and WMH

Intracranial (ICV), cerebrospinal fluid (CSF), brain tissue (BTV) and white matter hyperintensities (WMH) volumes were measured using Analyse 11.0 (http://analyzedirect.com) and in-house software 'MCMxxxVI', which is freely available from http://sourceforge.net/projects/bric1936/?source=directory. These methods which have been developed locally have been validated[256,261].

First, using the Object Extractor Tool in Analyse 11.0, the brain (i.e., all soft tissues including brain parenchyma, CSF, vessels and meninges within the inner skull and limited inferiorly by the tip of the odontoid peg at the foramen magnum) was extracted from the GRE scan. Next the region of interest (ROI) tool was used to manually correct erroneous structures and the final 'object' saved as the ICV. The volume of the ICV (in ml) was noted. Next, the GRE and FLAIR were fused in the red-green colour space (using the Register Tool in Analyse 11.0) for being used afterwards with MCMxxxVI for quantifying WMH volumes.

Using MCMxxxVI, CSF, venous sinuses and meninges, considered together as 'non-brain' intracranial features, were extracted. The software uses minimum variance quantisation[140] on the colour fused image from the prior step. The resultant 'non-brain' binary masks were subtracted from the ICV to provide a measure of BTV.

Finally, WMH were automatically extracted using MCMxxxVI, as described previously[140], then visually inspected for accuracy and to avoid erroneous classification, and falsities were manually corrected accordingly. The corrected WMH were considered final, and volumes in ml were recorded.

Prior to statistical analyses (e.g., correlation between WMH volumes and other data), the effect of head size was corrected for by dividing WMH volume by ICV volume. The WMH volume correlated strongly with the total Fazekas score (r = 0.83, $p<0.0001$).

Volumetric imaging measures – atrophy

In addition to the visual brain atrophy rating (see above), the BTV:ICV ratio was also used as a volumetric measure of atrophy; lower values reflect lower brain tissue volume. The BTV:ICV ratio correlated with the atrophy scores (deep $r = –0.72$, $p<0.0001$ and superficial $r = –0.74$, $p<0.0001$).

Tractography

The DT-MRI scans were used to assess white matter microstructural integrity by measuring mean diffusivity (MD) and fractional anisotropy (FA). All tractography analysis was performed blind to all other data. MRI data were converted from DICOM to NIfTI-1and pre-processed with FSL tools in order to extract the brain, eliminate bulk patient motion and eddy current-induced artefacts, and estimate the diffusion tensor parameters MD and FA. The underlying connectivity data was generated using a 2-fibre model and 5000 streamlines to reconstruct white matter tracts of interest.

Major white matter tracts were identified using probabilistic neighbourhood tractography (PNT), an automatic tract segmentation method based on modelling tract topology, as implemented in the TractoR package for fibre tracking analysis (http://www.tractor-mri.org.uk). PNT works by optimizing the choice of tractography seed point by estimating the best matching tract from a series of candidate tracts generated from a neighbourhood of native space voxels ($7 \times 7 \times 7$) placed around a voxel transferred from standard space against a pre-defined reference tract. The topological tract model is then used to reject any false positive connections, thereby significantly improving tract segmentation. The tracts assessed were the genu and

splenium of corpus callosum, bilateral corticospinal tracts, cingulum bundles, ventral cingulum bundles, left and right arcuate and inferior longitudinal fasciculi. For each subject, the seed point that produced the best match tract to the reference for each of the pathways was determined, with the resulting tractography mask applied to each subject's MD and FA volume. Tract-averaged MD and FA values, weighted by the connection probability, were calculated from these masks. To ensure that the segmented tracts were anatomically plausible, a researcher visually inspected all masks and excluded tracts with aberrant or truncated pathways.

Plasma markers

All participants had blood drawn on the day of MRI scanning, which provided data on levels of the pro-inflammatory cytokine interleukin-6 (IL-6), endothelial dysfunction (von Willebrand factor antigen (vWF Ag) and two measures of vWF activity: factor VIIIc (fVIIIc) and ristocetin cofactor (RCOF)), endothelial toxicity (homocysteine), cholesterol (total, high- (HDL) and low-density (LDL) lipoprotein) and anti-phospholipid antibodies (anti-cardiolipin Immunoglobulin G (IgG) and M (IgM)). Bloods were analysed in a fully-accredited, major NHS laboratory (http://www.edinburghlabmed.co.uk) that handles thousands of samples per day.

We also had access to blood data from recent clinic visits (most within one month, all within six months; and all analysed in the same lab) including SLE disease activity (C3, C4 and anti-double stranded DNA) and routine inflammatory markers (CRP and erythrocyte sedimentation rate (ESR)). Additionally, interferon beta (IFN) signatures were measured at the University of Manchester by analysis of messenger ribonucleic acid in a subset of 25 (49%) patients.

Statistical analysis

The distributions of relevant variables were checked graphically for normality (histograms and quantile-quantile plots) and presented as means (± SD) or medians (Q1–Q3) as appropriate. A p value of <0.05 was considered significant with the following caveat. In the interests of transparency, we report results from all analyses regardless of the p value as this aids interpretation of the entire study and we did not adjust the p values for multiple comparisons[262]. Thus, in some analyses, a p value of <0.01 or less might be more appropriately used for significance to account for multiplicity. All analyses were performed in R, version 3.0.1 (http://www.r-project.org/)[141]. Additional specific methods are noted hereunder.

SVD (results in Chapter 7)

We tested the association between the total SVD score and the Fazekas score and, separately, the WMH volume. We compared age and sex pairwise (SLE to healthy controls; SLE to stroke) by Student's *t* test and χ^2 test respectively. The individual features of SVD were compared for differences across the three groups by the Kruskal-Wallis test (a non-parametric analysis of variance) and a posttest comparison was used to identify the source of the difference.

We used ordinal logistic regression to test for associations between the total SVD score (range 0–4) and vascular risk factors (age, BMI, cholesterol and hypertension but not diabetes as no SLE patients had diabetes); plasma biomarkers of inflammation (IL6, ESR and CRP); endothelial dysfunction (vWF) and toxicity (homocysteine); rheumatology scores (SLEDAI-2K, BILAG and SLICC); SLE disease duration;

plasma markers of SLE activity (C3, C4 and anti-double stranded DNA); anti-phospholipid antibodies and brain atrophy. Results are presented as odds ratios (OR) with 95% CIs.

DT-MRI (results in Chapter 8)

Principal components analysis (PCA) was used to create general factors of MD and FA. PCA is a data reduction technique used to remove redundancy from several measured variables by replacing them with a smaller set of derived (latent) variables. PCA borrows concepts from linear algebra (such as eigenvalues, eigenvectors and singular value decomposition) in order to uncover hidden relationships in data that might otherwise not be obvious from other statistical techniques. Some measured variables contribute more weight to the general factor; this is assessed via inspection of each measurement's loading, i.e., its correlation with the general factor. Tract-averaged MD and FA were compared between SLE patients and healthy controls using Student's *t* test. We plotted MD and FA against age and tested for a difference in slope between patients and controls with an interaction term in a linear regression model. Mean fatigue in patients was compared with a normal reference range[58] using the Welch two-sample *t* test. Linear regression was used to examine the association between fatigue and other variables by univariate analyses. Multiple linear regression was used to adjust for age alone, then age plus disease duration plus current steroid use. PCA was also used to create a general factor of cognitive function (*g*)[263] from the three cognitive tools (MoCA, ACER, MMSE). Linear regression was used to examine the association between *g* and various potential explanatory variables by univariate analyses. Multiple linear regression was used to adjust for age alone, then age plus

disease duration plus current steroid use plus NART (to adjust for a person's prior (peak) cognitive function before the deleterious effect of age and SLE disease duration, if any). For all regression models, we checked multicollinearity, independence, constancy of variance and normality in the residuals.

Literature review and meta-analysis of DT-MRI studies (results in Chapter 8)

We searched MEDLINE and EMBASE (from 1990) on 15th January 2015 using the terms "diffusion-tensor imaging" and "diffusion-weighted imaging" in conjunction with the terms "lupus", "systemic lupus erythematosus" and "neuropsychiatric systemic lupus erythematosus". Acronyms and different combinations of the main search terms were also used. We checked reference lists of relevant papers for additional studies. We extracted data on study population (demographics, sample size, disease duration), imaging parameters, part of the brain measured (whole brain, specific tracts) and the reported diffusion measures. Standardised mean differences were calculated using the Cochrane Collaboration's Review Manager 5 software and used to compare DT-MRI findings in the meta-analysis.

Chapter 7: Cerebral small vessel disease burden is increased in SLE. Results and discussion from the SLE study

[submitted for publication]

Results

Subjects

Of 55 consecutive patients with SLE, 51 participated of mean age 48.8 ± 14.3 years (range 20 to 76 years), including 47 women (92%), and compared with 51 healthy controls (39 women (76%, p=0.06)) and 51 stroke patients (47 women, p=0.99). Of the four SLE patients that did not participate two had previous MRI claustrophobia and two gave no reason. Clinical data and blood results given in Tables 7.1 and 7.2.

Table 7.1 Subject charactersitics

	N (%) or mean ± SD or median (Q1–Q3)	**N (%) or mean ± SD or median (Q1–Q3)**	**p value**
	SLE (n=51)	Stroke (n=51)	
Females (n)	47 (92%)	47 (92%)	0.99
Age (years)	48.8 ± 14.3	55.3 ± 8.9	0.008
Disease duration (months)	50 (24–148)	NA	NA
Members of SLEx registry	31 (61%)	NA	NA
NPSLE	4 (8%)	NA	NA
BMI (kg / m2)	29 (6.5)	NA	NA
Current smoker	6 (12%)	28 (54.9%)	<0.0001
Hypertension	9 (18%)	32 (62.7%)	<0.0001
Diabetes	None	2 (3.9%)	NA
Past medical history of stroke	1 (1.9%)	51	NA
Current steroids	18 (35%)	NA	NA
Fatigue (score)	5.0 ± 1.7	NA	NA
MMSE (score)	28 (27–30)	NA	NA
MoCA (score)	26 (24–28)	NA	NA
ACER (score)	91 (87–94)	NA	NA
SLICC (score)	0 (0–1)	NA	NA
SLEDAI-2K (score)	2 (0–4)	NA	NA
BILAG (score)	2 (1–9)	NA	NA

ACER = Addenbrooke's Cognitive Examination – Revised, BMI = body mass index, BILAG = British Isles Lupus Assessment Group, MMSE = Mini Mental State Examination, MoCA = Montreal Cognitive Assessment, NPSLE = neuropsychiatric SLE, NA = not applicable, SLE = systemic lupus erythematosus, SLEx = Scottish Lupus Exchange Regitry, SLEDAI-2K = systemic lupus erythematosus Disease Activity Index 2000, SLICC = Systemic Lupus International Collaborating Clinics

Table 7.2 Blood results for 51 SLE patients

	N (%) or mean ± SD or median (Q1–Q3)	**Reference range**
Rheumatological		
C3 (mg / dL) (n=47)	1.2 ± 0.32	0.81–1.57
C4 (mg / dL) (n=47)	0.19 ± 0.09	0.13–0.39
Anti-ds-DNA (IU / mL) (n=47)	15 (8.5–33)	0–20
Inflammatory		
ESR (mm / hr) (n=49)	13 (6–21)	3–15
CRP (mg / L) (n=46)	2 (1–8)	0–5
IL-6 (pg / mL) (n=40)	1.72 (1.12–2.37)	
IFN (RQ value) (n=25)	6.7 (0.60–18.5)	
Endothelial dysfunction		
vWF Ag (IU / mL) (n=46)	1.71 ± 0.66	0.42–1.22
vWF fVIIIc (IU / mL) (n=46)	1.38 ± 0.45	0.5–1.5
vWF RCOF (IU / mL) (n=46)	1.37 ± 0.41	0.42–1.22
Homocysteine (umol / L) (n=45)	17 (15–21)	0–20
Lipids		
Total cholesterol (mmol / L) (n=49)	5.02 ± 0.95	<5.2
HDL cholesterol (mmol / L) (n=47)	1.42 ± 0.41	>1
LDL cholesterol (mmol / L) (n=46)	2.96 ± 0.84	<3.3
Antibodies		
Anti-cardiolipin IgG (GPL) (n=50)	3.35 (2.20–5.60)	0–13.3
Anti-cardiolipin IgM (MPL) (n=50)	1.65 (1.10–3.30)	0–9.8
Lupus anticoagulant (n=47)	5 positive	Positive/Negative
Anti-ds-DNA = anti-double stranded DNA, CRP = C-reactive protein, ESR = erythrocyte sedimentation rate, HDL = high density lipoprtein, IFN = interferon, IgG = immunoglobulin isotype G, IgM = immunoglobulin isotype M, IL-6 = interleukin-6, LDL = low density lipoprotein, vWF Ag = von Willebrand factor antigen, vWF fVIIIc = von Willebrand factor factor VIIIc, vWF RCOF = von Willebrand factor ristocetin co-factor		

Healthy controls were of similar age (44.9 ± 11.1 years, p=0.12) while the stroke patients were on average six years older (55.3 ± 8.9 years, p=0.008). Four SLE patients had neuropsychiatric SLE (NPSLE), six were current smokers, nine had hypertension, none had diabetes and one had a prior ischaemic stroke. Eighteen were prescribed steroids at the time of assessment. There were significantly more smokers and hypertensives in the stroke group. The inflammatory markers ESR and CRP were raised in 22/49 (45%) and 17/45 (38%) of SLE patients versus these tests' normal reference ranges. Homocysteine was raised in 37/45 (82%) SLE patients.

WMH, PVS, lacunes and microbleeds

Periventricular and deep WMHs were seen in 49/51 (96%) and 36/51 (70%) SLE patients respectively. All SLE patients had visible PVS. Lacunes were seen in five (10%) and microbleeds in two (4%) SLE patients.

SVD imaging biomarkers in SLE versus healthy controls and stroke patients

Compared to healthy controls, SLE patients had a greater total SVD score (Table 7.3) sustained across each 10-year age band (Figure 7.1), including more deep but not periventricular WMHs. Compared to stroke patients, the SLE patients also had higher SVD score, mostly due to having more PVS, and similar deep and periventricular WMHs. SLE patients had more superficial, but not deep, atrophy versus healthy controls. There was no difference in either deep or superficial atrophy score between SLE and stroke patients. Individual SVD features by age are given in Figure 7.2.

Table 7.3 Imaging biomarkers of SVD in SLE patients, healthy controls and stroke patients

	Group			**Kruskal-Wallis**	**Pairwise posttest p value**		
	SLE (n=51)	Stroke (n=51)	Controls (n=51)	Group p value	SLE – Stroke	SLE – Controls	Stroke – Controls
Lacunes	0 (0–2)	0 (0–3)	0 (0–0)	0.02			0.01
Microbleeds	0 (0–1)	0 (0–15)	0 (0–0)	0.15			
PVS BG (score 0–4)	2 (1–4)	1 (0–3)	1 (1–2)	<0.0001	<0.0001	<0.0001	
PVS CS (score 0–4)	3 (1–4)	1 (0–4)	1 (0–3)	<0.0001	<0.0001	<0.0001	0.001
WMH periventricular (score 0–3)	1 (0–3)	1 (0–3)	1 (1–2)	0.48			
WMH deep (score 0–3)	1 (0–3)	1 (0–3)	0 (0–2)	<0.0001		0.01	0.0007
WMH (total Fazekas score 0–6)	2 (1–6)	2 (0–6)	1 (1–4)	0.02		0.05	0.03
Total SVD score (score 0–4)	**1 (0–3)**	**0 (0–4)**	**0 (0–2)**	**<0.0001**	**0.02**	**<0.0001**	**0.0006**
Deep atrophy (score 1–6)	1 (1–5)	1 (1–4)	1 (1–4)	0.14			
Superficial atrophy (score 1–6)	1 (1–4)	1 (1–3)	1 (1–4)	0.006		0.004	

Values are medians (range). The p value compares the individual SVD features across the three groups by Kruskal-Wallis test. Posttest comparisons identifies where differences exists. Blank cells = non-sgnificant pairwise relationship. BG = basal ganglia, CS = centrum semiovale, PVS = perivascualr spaces, SVD = small vessel disease, SLE = systemic lupus erythematosus, WMH = white matter hyperintensities.

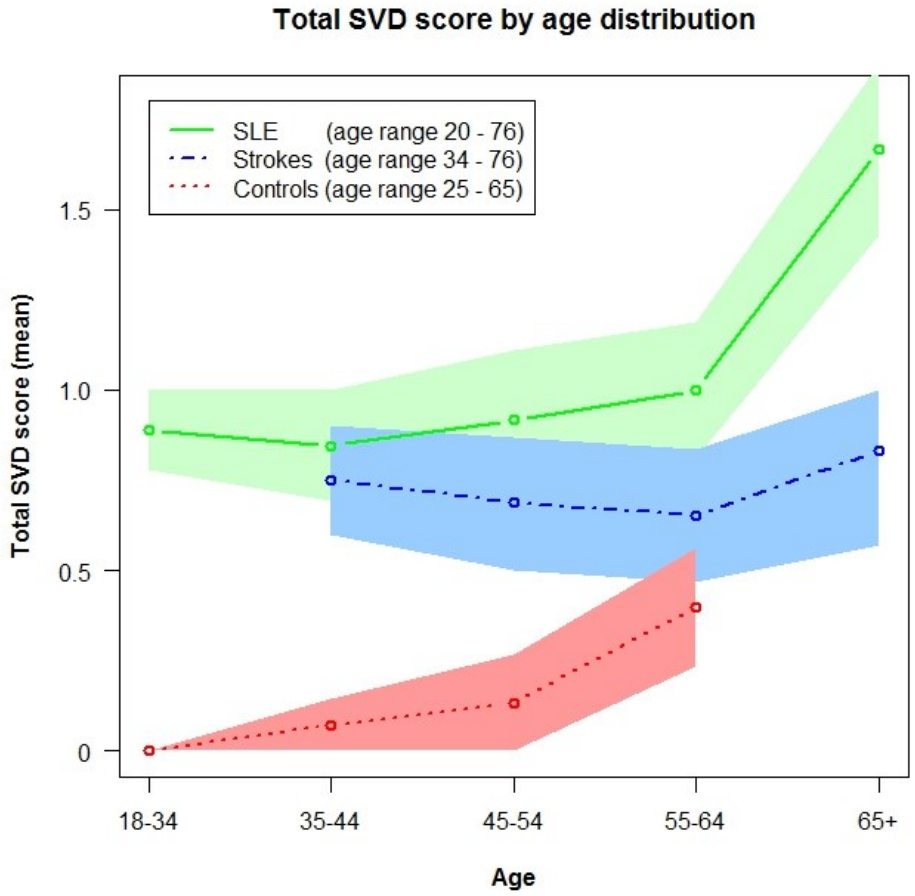


Figure 7.1 Total SVD score (mean plus 95% CI) by age distribution in SLE, stroke and healthy controls

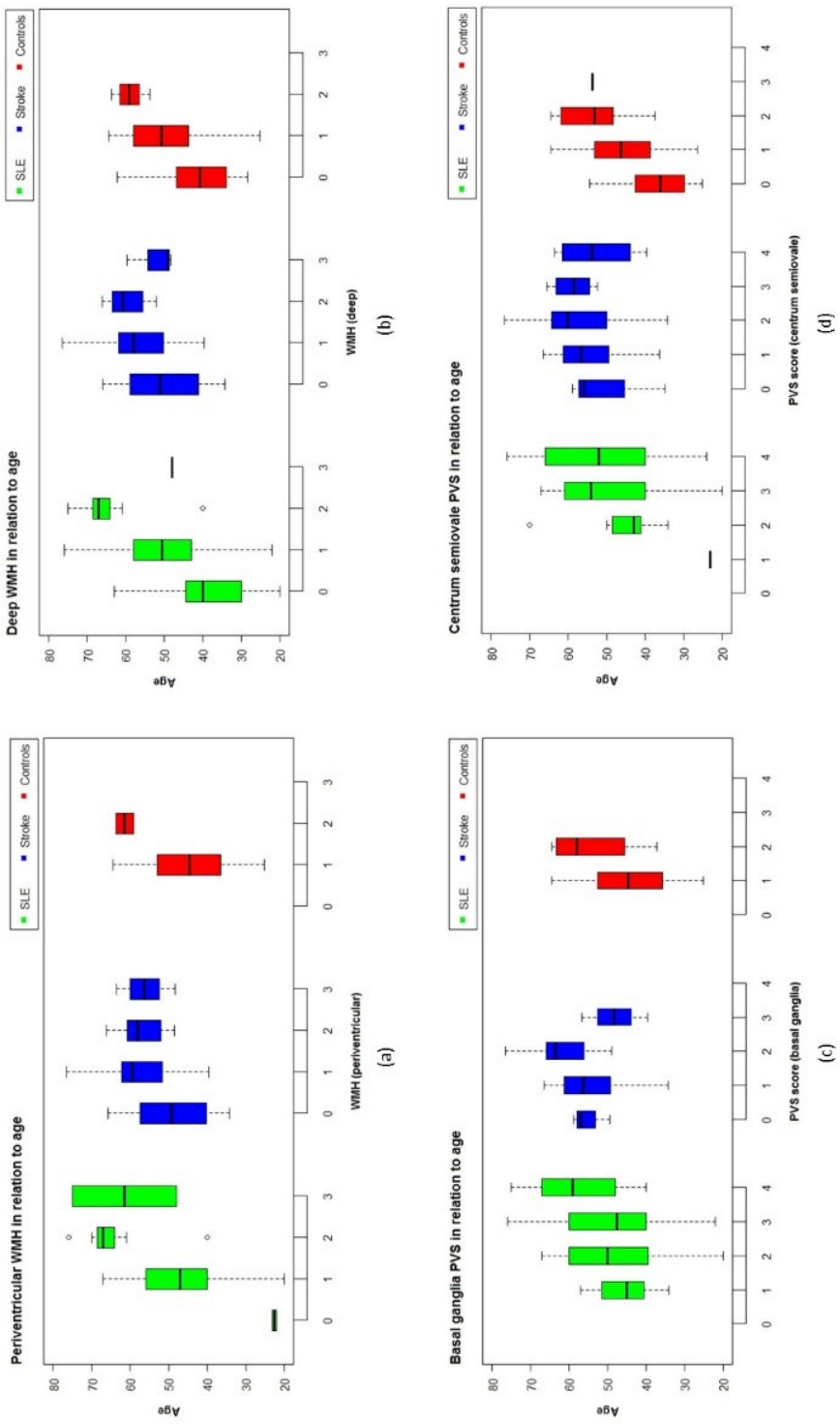


Figure 7.2 Scores of individual features of SVD in (a) periventricular WMH, (b) deep WMH, (c) basal ganglia PVS and (d) and centrum semiovale PVS by age among SLE, stroke and healthy controls

Association between total SVD score and other variables

In SLE, the total SVD score was associated with age (OR 1.05, 95% CI 1.01–1.09), hypertension (1.82, 1.13–2.93), higher levels of MD (2.58, 1.32–5.06) and lower levels of FA (0.42, 0.22–0.80). The association with hypertension did not remain after adjusting for age (Table 7.4). Higher SVD burden was inversely associated with fatigue (older people had less fatigue) but not when adjusted for age. The total SVD score was not associated with SLE activity (by SLE activity scoring tools or blood markers of activity), accumulated damage (SLICC) nor SLE disease duration. The total SVD score was not associated with CRP. However, we excluded one SLE patient from our analysis of CRP as they reported a tooth infection in the week prior to their scan (CRP of 155, i.e., a considerable outlier). When this patient was included, CRP was associated with total SVD score (1.05, 1.01–1.09), but since this result depends on one patient's data it is not reliable. More PVS in the centrum semiovale were associated with higher levels of HDL cholesterol (14.88, 2.76–80.09; Table 7.5) which remained significant after adjusting for age and BMI (16.99, 2.98–96.66). No other individual SVD feature showed significant associations with other variables (vascular risk factors, SLE activity or blood markers) (PVS and WMH shown in Table 7.5).

NPSLE

Patients diagnosed with NPSLE (n=4) had more deep WMH compared with SLE (p=0.04, Table 7.6) but data were limited.

Table 7.4 Univariate and multivariable associations between total SVD score and other variables in SLE patients

	Unadjusted		Age adjusted		Adjusted for age and SLE disease duration	
	OR	95% CI	OR	95% CI	OR	95% CI
Vascular risk factors						
Age (years)	1.05	1.01 – 1.09 *	--	--	1.05	1.00 – 1.10 *
Hypertension (classified)	1.82	1.13 – 2.93 *	1.58	0.95 – 2.63	1.58	0.95 – 2.63
BMI (kg / m2)	0.97	0.89 – 1.06	0.95	0.87 – 1.04	0.95	0.87 – 1.04
Disease duration (months)	1.00	0.99 – 1.01	1.00	0.99 – 1.00	--	--
Current smoker (Yes/No)	0.91	0.15 – 5.29	1.19	0.19 – 7.32	1.22	0.19 – 7.55
Ever smoked (Yes/No)	2.06	0.63 – 6.80	2.00	0.67 – 6.62	2.01	0.60 – 6.76
Steroids (Yes/No)	0.63	0.18 – 2.16	0.62	0.18 – 2.14	0.62	0.18 – 2.13
Neurological						
Fatigue	0.68	0.47 – 0.98 *	1.04	0.99 – 1.08	1.00	0.99 – 1.00
Anxiety	0.97	0.87 – 1.08	1.02	0.90 – 1.14	1.02	0.90 – 1.14
Depression	0.97	0.85 – 1.09	1.01	0.88 – 1.15	1.01	0.88 – 1.15
MMSE	0.94	0.63 – 1.38	1.05	0.69 – 1.58	1.07	0.69 – 1.67
MoCA	1.06	0.89 – 1.26	1.11	0.92 – 1.33	1.12	0.93 – 1.36
ACER	1.01	0.94 – 1.09	1.02	0.95 – 1.11	1.03	0.95 – 1.12
Rheumatology scores						
SLEDAI-2K	1.01	0.78 – 1.30	1.01	0.78 – 1.31	1.01	0.78 – 1.31
BILAG	0.95	0.85 – 1.07	0.98	0.87 – 1.11	0.99	0.87 – 1.11
SLICC	1.14	0.68 – 1.91	0.93	0.53 – 1.62	0.88	0.48 – 1.65
DTI biomarkers						
MD across 12 tracts (n=47)	2.58	1.32 – 5.06 **	2.32	1.16 – 4.64 *	2.53	1.22 – 5.22 *
FA across 12 tracts (n=47)	0.42	0.22 – 0.80 **	0.47	0.24 – 0.93 *	0.43	0.21 – 0.88 *
Bloods						
C3 (mg / dL) (n=47)	1.06	0.15 – 7.56	1.06	0.14 – 8.11	1.14	0.14 – 8.93
C4 (mg / dL) (n=47)	1.07	0.52 – 2.20	1.14	0.55 – 2.35	1.17	0.56 – 2.47
Anti-ds-DNA (IU / mL) (n=47)	0.99	0.98 – 1.01	0.99	0.98 – 1.00	0.99	0.98 – 1.01
ESR (mm / hr) (n=49)	1.03	0.99 – 1.06	1.02	0.98 – 1.06	1.02	0.98 – 1.06
CRP (mg / L) (n=45)	0.99	0.92 – 1.08	0.99	0.92 – 1.08	1.00	0.92 – 1.08
IL-6 (pg / mL) (n=40)	0.99	0.71 – 1.40	1.02	0.72 – 1.43	1.02	0.72 – 1.43
IFN (RQ value) (n=24)	1.04	0.94 – 1.14	1.04	0.95 – 1.14	1.04	0.93 – 1.16
vWF Ag (IU / mL) (n=46)	1.21	0.48 – 3.00	0.88	0.33 – 2.30	0.87	0.33 – 2.31
vWF F8c (IU / mL) (n=46)	2.50	0.66 – 9.45	2.30	0.62 – 8.57	2.31	0.62 – 8.61
vWF RCOF (IU / mL) (n=46)	2.00	0.46 – 8.56	1.24	0.26 – 5.83	1.25	0.26 – 5.91
Homocysteine (umol / L) (n=45)	1.03	0.94 – 1.12	1.01	0.92 – 1.11	1.01	0.92 – 1.11
Tot cholesterol (mmol / L) (n=49)	1.55	0.84 – 2.87	1.37	0.72 – 2.59	1.38	0.73 – 2.62
HDL cholesterol (mmol / L) (n=47)	1.35	0.32 – 5.74	1.41	0.33 – 6.04	1.39	0.32 – 6.02
LDL cholesterol (mmol / L) (n=46)	1.72	0.85 – 3.49	1.47	0.71 – 3.01	1.50	0.71 – 3.14
Anti-cardiolipin IgG (GPL) (n=50)	0.92	0.77 – 1.09	0.93	0.78 – 1.11	0.93	0.78 – 1.10
Anti-cardiolipin IgM (MPL) (n=50)	1.00	0.95 – 1.06	0.98	0.93 – 1.04	0.98	0.93 – 1.04
Lupus anticoagulant (n=47)	0.42	0.06 – 2.73	0.30	0.04 – 2.18	0.30	0.04 – 2.15

* $p<0.05$, ** $p<0.01$

ACER = Addenbrooke's Cognitive Examination – Revised, BMI = body mass index, BILAG = British Isles Lupus Assessment Group, BTV = brain tissue volume, CRP = C-reactive protein, DTI = diffusion tensor imaging, ESR = erythrocyte sedimentation rate, FA = fractional anisotropy, HDL = high density lipoprotein, LDL = low density lipoprotein, MD = mean difussivity, MMSE = Mini Mental State Examination, MoCA = Montreal Cognitive Assessment, IFN = interferon beta, IL-6 = interleukin-6, ICV = intracranial volume, SLEDAI-2K = systemic lupus erythematosus Disease Activity Index, SLICC = Systemic Lupus International Collaborating Clinics, vWF Ag = von Willebrand factor antigen, vWF F8c = von Willebrand factor VIII, vWF RCOF = von Willebrand factor ristocen co-factor

Table 7.5 Unadjusted univariate associations between individual SVD features and other variables in SLE patients

	PVS BG		PVS CS		WMH (Total Fazekas)	
	OR	95% CI	OR	95% CI	OR	95% CI
Vascular risk factors						
Age (years)	1.02	0.98 – 1.06	1.03	0.99 – 1.07	1.13	1.07 – 1.20 ***
Hypertension (classified)	1.36	0.90 – 2.05	1.25	0.84 – 1.86	1.88	1.20 – 2.94 **
BMI (kg / m2)	0.97	0.89 – 1.05	0.98	0.91 – 1.07	1.03	0.95 – 1.25
Disease duration (months)	1.00	0.99 – 1.00	1.00	0.99 – 1.00	1.00	0.99 – 1.00
Current smoker (Yes/No)	0.87	0.19 – 3.92	0.50	0.11 – 2.34	1.87	0.37 – 9.40
Ever smoked (Yes/No)	0.82	0.30 – 2.23	0.77	0.27 – 2.18	2.63	0.87 – 7.89
Steroids (Yes/No)	0.48	0.17 – 1.41	0.74	0.25 – 2.24	1.44	0.47 – 4.36
Rheumatology scores						
SLEDAI-2K	1.10	0.88 – 1.37	1.04	0.80 – 1.35	1.00	0.78 – 1.29
BILAG	0.96	0.86 – 1.07	0.93	0.84 – 1.04	0.96	0.86 – 1.07
SLICC	0.86	0.55 – 1.35	0.92	0.57 – 1.49	1.42	0.88 – 2.28
Bloods						
C3 (mg / dL) (n=47)	0.35	0.06 – 1.98	1.08	0.22 – 5.29	0.70	0.13 – 3.71
C4 (mg / dL) (n=47)	0.85	0.45 – 1.62	1.16	0.64 – 2.09	0.79	0.42 – 1.49
Anti-ds-DNA (IU / mL) (n=47)	1.00	0.99 – 1.02	1.00	0.99 – 1.01	1.00	0.99 – 1.01
ESR (mm / hr) (n=49)	1.02	0.98 – 1.05	0.99	0.96 – 1.02	1.03	0.99 – 1.06
CRP (mg / L) (n=45)	1.00	0.99 – 1.03	1.02	0.98 – 1.06	1.02	0.99 – 1.04
IL-6 (pg / mL) (n=40)	1.04	0.79 – 1.37	0.94	0.71 – 1.24	0.98	0.74 – 1.29
IFN (RQ value) (n=24)	0.93	0.86 – 1.00	0.97	0.91 – 1.05	0.98	0.90 – 1.06
vWF Ag (IU / mL) (n=46)	0.96	0.43 – 2.17	1.56	0.67 – 3.60	1.30	0.56 – 2.99
vWF F8c (IU / mL) (n=46)	1.39	0.40 – 4.84	2.58	0.69 – 9.64	1.89	0.54 – 6.63
vWF RCOF (IU / mL) (n=46)	1.07	0.29 – 3.89	1.62	0.42 – 6.21	1.34	0.31 – 5.76
Homocysteine (umol / L) (n=45)	0.97	0.89 – 1.04	1.02	0.94 – 1.11	1.07	0.98 – 1.16
Tot cholesterol (mmol / L) (n=49)	1.75	0.98 – 3.12	1.40	0.80 – 2.46	1.31	0.73 – 2.35
HDL cholesterol (mmol / L) (n=47)	3.30	0.86 – 12.6	14.8	2.76 – 80.0 ***	0.95	0.23 – 3.99
LDL cholesterol (mmol / L) (n=46)	1.89	0.96 – 3.71	1.11	0.58 – 2.12	1.38	0.70 – 2.73
Anti-cardiolipin IgG (GPL) (n=50)	1.00	0.86 – 1.16	0.94	0.80 – 1.09	0.88	0.75 – 1.04
Anti-cardiolipin IgM (MPL) (n=50)	1.02	0.97 – 1.08	1.02	0.97 – 1.07	0.99	0.94 – 1.04
Lupus anticoagulant (n=47)	0.79	0.14 – 4.67	1.23	0.22 – 6.84	0.60	0.11 – 3.22

** p<0.01 *** p<0.0001

BMI = body mass index, BILAG = British Isles Lupus Assessment Group, BTV = brain tissue volume, CI = confidence interval, CRP = C-reactive protein, DTI = diffusion tensor imaging, ESR = erythrocyte sedimentation rate, HDL = high density lipoprotein, LDL = low density lipoprotein, IFN = interferon beta, IL-6 = interleukin-6, ICV = intracranial volume, OR = odds ratio, PVS BG = perivascular spaces in basal ganglia, PVS BG = perivascular spaces in centrum semiovale, SLEDAI-2K = systemic lupus erythematosus Disease Activity Index, SLICC = Systemic Lupus International Collaborating Clinics, vWF Ag = von Willebrand factor antigen, vWF F8c = von Willebrand factor VIII, vWF RCOF = von Willebrand factor ristocen co-factor, WMH = white matter hyperintensties

Table 7.6 Imaging biomarkers of SVD in SLE and NPSLE patients

	Median (Q1–Q3)	**Median (Q1–Q3)**	**p value**
	SLE	NPSLE	
N	47	4	
Lacunes	0 (0–0)	0 (0–0)	0.52
Microbleeds	0 (0–0)	0 (0–0)	0.71
PVS BG (score 0–4)	2 (2–3)	2.5 (1.5–3.0)	0.76
PVS CS (score 0–4)	3 (3–4)	3.0 (1.5–4.0)	0.72
WMH periventricular (score 0–3)	1 (1–1)	1.5 (0.5–2.5)	0.43
WMH deep (score 0–3)	1 (0–1)	1.5 (1.0–2.5)	0.04 *
WMH (total Fazekas score 0–6)	2 (1–2)	3.0 (1.5–5.0)	0.22
Total SVD score (score 0–4)	**1 (1–2)**	**1.5 (1.0–3.5)**	**0.43**
Deep atrophy (score 1–6)	1 (1–2)	1.5 (1.0–2.5)	0.49
Superficial atrophy (score 1–6)	1 (1–1)	1.5 (0.5–2.0)	0.59

* $p<0.05$
BG = basal ganglia, CS = centrum semiovale, NPSLE = neurpsychiatric SLE, PVS = perivascualr spaces, SVD = small vessel disease, SLE = systemic lupus erythematosus, WMH = white matter hyperintensities.

Discussion

We show that patients with SLE have more SVD markers, notably PVS and deep WMH, than sex- and age-matched healthy controls and mild stroke patients from the same health region. Deep WMH were worse in four patients with NPSLE. Our patients were not selected on the basis of neurological involvement and only 4/51 were diagnosed with NPSLE. There were more lacunes, higher PVS and WMH scores in SLE patients versus healthy controls. Moreover, despite the stroke patients being slightly older with far more smokers and hypertensives, the SLE patients had more PVS and an equal burden of WMH. The higher burden of SVD, particularly PVS as a marker of inflammation, provides a possible explanation for increased stroke risk and suggests that systemic inflammation is associated with microvascular brain damage in SLE patients even in the absence of neurological symptoms even though there was no association between SVD markers and plasma inflammatory markers or disease duration, activity and damage scores.

Several cross-sectional studies (reviewed in Chapter 4), including ~1,200 SLE patients reported features of SVD, such as WMHs and atrophy, but many of these studies focused on NPSLE patients, while few compared SLE to healthy controls (instead most compared SLE to NPSLE, or SLE to patients with antiphospholipid syndrome), none compared SLE to mild stroke patients which allows for comparison with clinically overt SVD, and none included the range of SVD features assessed here with validated scoring tools. Longitudinally, a 20-year MRI follow-up study showed increased number and volume of WMHs and brain volume loss in most of 30 SLE patients studied, but may have reflected mainly ageing effects[264]. In a shorter follow-up study of 75 SLE patients, predictors of new or increased WMH included

antiphospholipid antibodies, SLE damage scores and higher dose of corticosteroids and there was more grey and white matter volume loss versus controls[206,207].

PVS on neuroimaging (in SLE or any other cohort) could reflect inflammatory activity[265]. PVS are not normally seen on brain imaging with MRI at 1.5 Tesla (as used in our study) but we note the higher spatial resolution of higher field strength MRI scanners will increase detection and complicate interpretation, until such time when sufficient control data from the normal population becomes available. This could also complicate assessment of associations with inflammation. PVS were associated with the inflammatory marker CRP in a large cohort (n=634) of community-dwelling older people (ß=0.12, p=0.048)[48]. In our study, the total SVD score and PVS were not associated with any blood measure of inflammation (ESR, CRP, IL-6) or with clinical SLE disease activity or disease burden score. However, we note about 40% of patients had raised ESR and CRP. Moreover, our cross-sectional study design did not permit us to associate inflammatory flares over time with the evolution, or not, of PVS. We did not select patients on the basis of SLE activity and cannot exclude the possibility that blood markers of inflammation (captured during a flare-up) will associate with PVS in a larger or longitudinal study. Chung et al. also found no association between systemic inflammation (measured using a novel marker, GlycA) and SLE activity, despite the presence of systemic inflammation[266].

Perivascular inflammation of the small cerebral vessels is a prominent finding in SLE[267] at autopsy as well as in sporadic SVD[46]. Some studies have noted PVS[208,268] on brain imaging in SLE patients (n=122), but data are limited and none compared the total PVS load with a non-SLE comparator group. In a recent, but smaller (n=11), post-mortem study of vascular changes in SLE, a third of subjects had

microthromboemboli, glial hyperplasia, neuronal loss, microaneurysms, lacunar infarcts and microbleeds which correlated with neuroimaging, including recent subcortical infarcts, lacunes, WMHs and atrophy; stroke and cognitive impairment were more frequent findings amongst these patients compared to the SLE patients that did not have histology evidence of SVD[269].

We note an association between higher levels of HDL cholesterol and more PVS in the centrum semiovale which remained significant after adjusting for age and BMI. Additionally, there was a trend towards an association between total, HDL and LDL cholesterol and PVS in the basal ganglia (p values 0.05 to 0.07). High HDL cholesterol is traditionally considered protective against cardiovascular disease including ischaemic stroke, but a recent meta-analysis showed that drugs designed to boost HDL had no effect on improving cardiovascular outcomes[270]. Additionally, a gene variation in some people impairs HDL uptake, making them susceptible to cardiovascular disease despite high HDL[271]. In a cohort study[272] of 210 SLE patients followed for 29 months, functional HDL (a novel marker of inflammation) was associated with carotid plaques (OR 9.1, 95% CI 3.3–24.6). Meanwhile, higher HDL is associated with increased risk of haemorrhagic stroke in the general population (relative risk 1.17 (95% CI 1.02–1.35); 7,960 strokes, 1.4M participants)[273]. The reason why cholesterol might relate to PVS in SLE is unknown and could be spurious given our study's lack of power, but HDL can become dysfunctional resulting in inflammation[274,275] and endothelial dysfunction[276].

The inverse relationship between higher SVD burden and lower levels of fatigue was explained by age – the older SLE patients (that had more SVD features) were less fatigued.

Our data further validates the concept of a 'total SVD score'[258,259] as a simple surrogate marker for total brain damage due to SVD. The association of deep and superficial atrophy on univariate analysis, but not in adjusted analyses, is in agreement with Staals et al.[258] and suggests atrophy should remain complementary, but not core, in the assessment of SVD as atrophy co-associates with age. Our volume measure of atrophy (the BTV:ICV ratio) was also associated with total SVD burden. Hypertension was also associated with the total SVD score, but, unlike Staals et al.,[258] smoking (current or ever) was not, although our study was underpowered and only 12% of our patients smoked compared with a third of theirs.

NPSLE patients had more deep WMH than SLE patients (as expected since NPSLE implies more clinically overt brain damage), but the number of patients with NPSLE in our study was small which limits generalizability.

Further work

Larger longitudinal studies are needed to fully appreciate the significance of SVD in SLE, for example to elucidate the contribution of SLE activity, diet and lifestyle, SLE treatments or some other variable in causing accelerated brain damage in these patients. This pilot study clearly demonstrates the feasibility of such a study. Of 55 consecutive patients invited to participate, 51 agreed to take part (93% uptake). All the participants tolerated the MRI scanner environment (there were no cases of claustrophobia among the 51 participants that took part in the study) and completed the full exam (~50 mins). Venepuncture failed in a handful of participants. Our MRI sequences (including the diffusion tensor sequences (see next Chapter) and methods for image analysis could be used in a larger multi-centre study.

Chapter 8: Fatigue and cognitive function in SLE: associations with white matter microstructural damage. A diffusion tensor MRI study, literature review and meta-analysis. Results and discussion from the SLE study

[submitted for publication]

Results

Subjects

Fifty-one patients with SLE were recruited of mean age 48.8 ± 14.3 years (range 20 to 76 years), including 47 women (92%) which is consistent with community prevalence. Clinical, fatigue and cognitive data are given in Table 8.1. MD, FA and NART scores were available for 51 age- and sex-matched healthy controls of mean age 44.9 ± 11.1 years, including 39 women (76%). A third of patients (18/51 (35%)) were currently prescribed corticosteroids.

Table 8.1 Subject charactersitics

SLE patients, n = 51	**N (%) or mean ± SD or median (Q1–Q3)**	**Reference data from controls or standard ranges**
Female	47 (92%)	39 (76%); p=0.06
Age (years)	48.8 ± 14.3	44.9 ± 11.1; p=0.12
Disease duration (months)	50 (24.5–148)	
Members of SLEx registry	31 (61%)	
NPSLE	4 (8%)	
BMI (kg / m2)	29 (6.5)	20-25 normal, 25-30 overweight, 30-35 obese, 35+ clinically obese
Current smoker	6 (12%)	
Hypertension	9 (18%)	≥140/90 mm Hg
Fatigue (score)	5.0 ± 1.7	2.3 ± 0.7; p<0.0001
Anxiety (score)	6 (3–12)	0-7 non-case, 8-10 borderline, 11+ case
Depression (score)	8 (6–12)	0-7 non-case, 8-10 borderline, 11+ case
Current steroids	18 (35%)	
MoCA (score) (n=50)	26 (24–28)	0–30. Normal ≥26
ACER (score) (n=50)	91 (87–94)	0–100. Normal ≥88
MMSE (score) (n=50)	28 (27–30)	0–30. Normal ≥27
NART (score) (n=50)	34 (27–39)	40 (34–42); p=0.0008

ACER = Addenbrooke's Cognitive Examination – Revised, BMI = body mass index, MoCA = Montreal Cognitive Assessment, MMSE = Mini Mental State Examination, NART = National Adult Reading Test, SLE = systemic lupus erythematosus, SLEx = Scottish systemic lupus erythematosus exchange registry

General factors for MD and FA

DT-MRI failed in four patients so the analyses are based on 47 patients. In SLE, the general factors MD and FA accounted for 40% and 27% of the variance among tracts, respectively. A similar level of variance was explained in the control group. Factor loadings of each tract are given in Table 8.2. An image illustrating whole brain white matter tractography from a representative patient along with a group map illustrating two tracts (the genu and splenium of corpus callosum) is shown in Figure 8.1.

Table 8.2 Factor loadings for white matter tracts

	SLE (n=47)		**Controls (n=47)**	
	MD	**FA**	**MD**	**FA**
Genu	0.68	0.61	0.68	0.33
Splenium	0.01	-0.34	0.22	0.21
Left cingulum	0.77	0.67	0.73	0.76
Right cingulum	0.87	0.60	0.79	0.76
Left CST	0.73	0.55	0.65	0.34
Right CST	0.43	0.56	0.77	0.18
Left ILF	0.76	0.48	0.57	0.64
Right ILF	0.28	0.08	0.19	0.40
Proportion of shared variance	0.40	0.27	0.38	0.25
CST = corticospinal tract, FA = fractional anisotropy, MD = mean diffusivity, ILF = inferior longitudinal fasciculus				

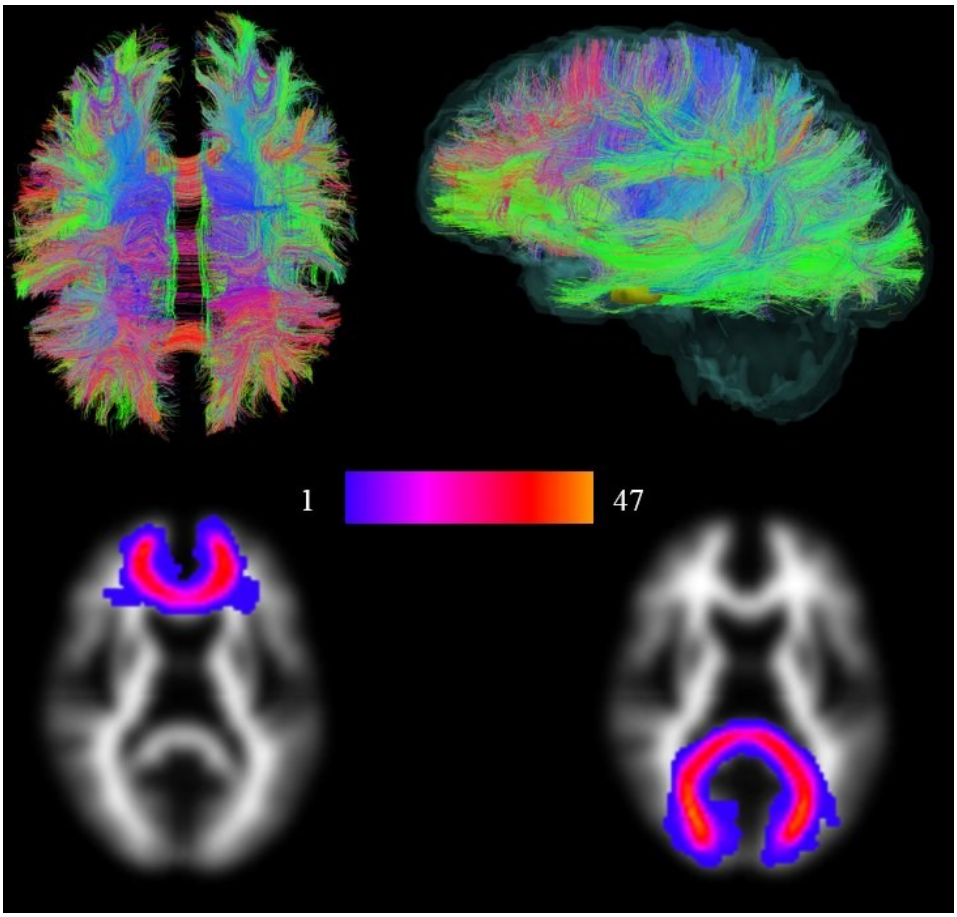


Figure 8.1 Whole brain tractography and two major tracts. The top row shows maps of whole brain white matter structure from a representative patient. White matter tracts running predominantly anterior/posterior are coloured green, superior/inferior blue and right/left red. The lower row shows group maps (all patients superimposed on each other) of two major tracts, the genu (left image) and splenium (right) of corpus callosum for all 47 patients. Note the close correspondence of these tracts, indicated by the lighter red/yellow colours, which allows the integrity of the same structure to be measured across the cohort.

Comparing MD and FA to healthy controls

MD was significantly higher in all tracts ($p<0.0001$ for all tracts) in SLE patients versus controls (Table 8.3). FA was significantly higher in SLE patients than controls in the genu ($p<0.0001$) and left corticospinal tract ($p<0.0001$), and significantly lower in the splenium ($p=0.008$), and left ($p=0.04$) and right ($p=0.01$) cingulum bundles (Table 8.3). Overall, the expected increase in MD and decrease in FA with age appeared accelerated in SLE patients versus controls (Figure 8.2). However, the patients' and controls' regression slopes (for both MD and FA) did not differ when tested via an interaction term in a linear regression model.

Table 8.3 Comparison of MD and FA values for white matter tracts between SLE patients and healthy controls

	SLE (n=47)	**Controls (n=47)**	**p value**	**Cohen's d**
Female n (%)	43 (91.5%)	41 (87.2%)	p=0.74	NA
Age (years)	48.5 ± 13.7	44.6 ± 11.5	p=0.14	NA
MD (10^{-6} mm^2 /s)				
Genu	833.18 ± 68.13	751.29 ± 57.36	p<0.0001	1.33
Splenium	1072.33 ± 162.98	761.49 ± 129.37	p<0.0001	2.19
Left cingulum	740.69 ± 44.08	624.21 ± 35.41	p<0.0001	3.01
Right cingulum	721.06 ± 43.33	619.70 ± 35.74	p<0.0001	2.62
Left CST	729.25 ± 30.76	667.24 ± 39.4	p<0.0001	1.82
Right CST	740.96 ± 30.18	683.32 ± 35.31	p<0.0001	1.79
Left ILF	781.68 ± 49.53	728.42 ± 66.48	p<0.0001	0.96
Right ILF	818.16 ± 136.72	703.82 ± 63.03	p<0.0001	1.29
Average	804.6 ± 39.8	692.4 ± 32.2	p<0.0001	3.20
FA (unitless, values lie in the range 0 to 1)				
Genu	0.49 ± 0.06	0.42 ± 0.04	p<0.0001	1.42
Splenium	0.52 ± 0.07	0.55 ± 0.06	p=0.008	-0.57
Left cingulum	0.44 ± 0.05	0.46 ± 0.04	p=0.04	-0.45
Right cingulum	0.42 ± 0.04	0.44 ± 0.03	p=0.01	-0.57
Left CST	0.49 ± 0.04	0.44 ± 0.04	p<0.0001	1.26
Right CST	0.47 ± 0.04	0.48 ± 0.04	p=0.24	-0.25
Left ILF	0.45 ± 0.05	0.43 ± 0.04	p=0.08	0.36
Right ILF	0.42 ± 0.05	0.43 ± 0.04	p=0.59	-0.11
Average	0.46 ± 0.02	0.46 ± 0.02	p=0.25	0.24
CST = corticospinal tract, FA = fractional anisotropy, ILF = inferior longitudinal fasciculus, MD = mean diffusivity.				

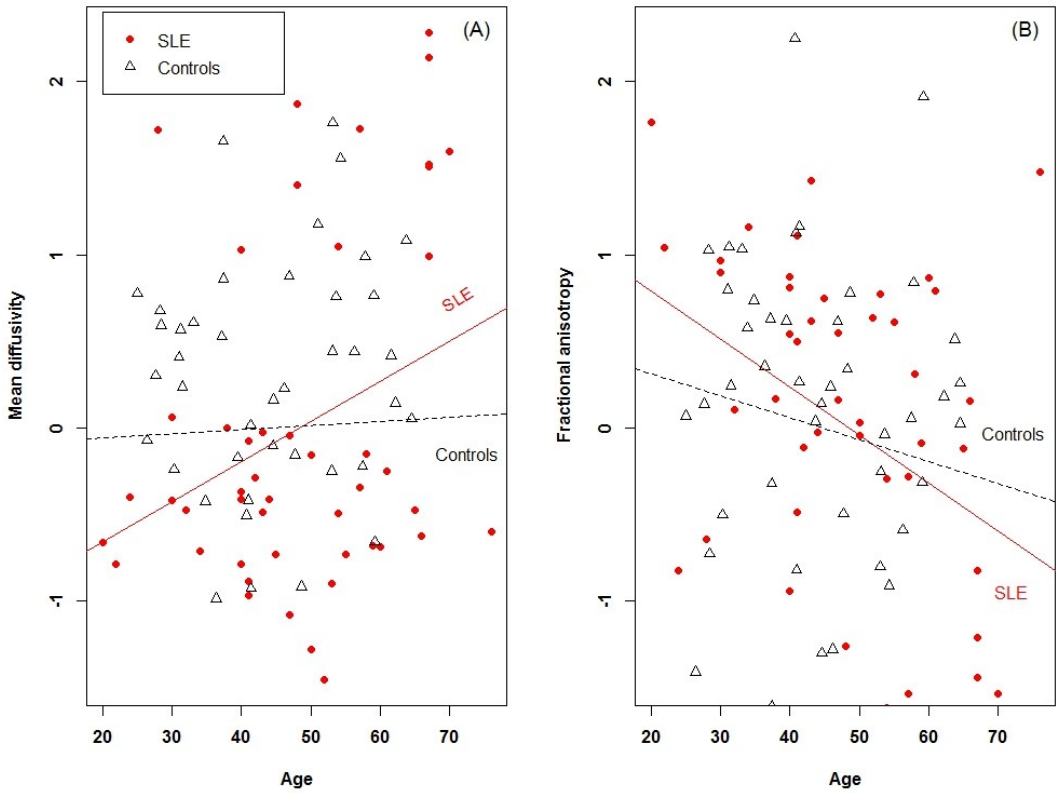


Figure 8.2 General factors for (A) mean diffusivity and (B) fractional anisotropy in relation to age among SLE patients and healthy controls

MD and FA – associations with other variables

In univariate analyses, higher MD was associated with older age (r = 0.32, p=0.03), less fatigue (r = –0.35, p=0.01), more brain atrophy (all three measures of atrophy, see Table 8.4), more accumulated SLE damage on SLICC scoring (r = 0.32, p=0.03), worse performance on MoCA (r = –0.30, p=0.04) and poorer general cognitive function (g) (r = –0.31, p=0.04) (Table 8.4). MD was not associated with any of the plasma markers (Table 8.5). The association between higher MD and SLICC damage

did not remain when adjusted for age. The association between higher MD and cognitive function (MoCA and *g*) remained after adjustment for age.

In univariate analyses, *lower* FA was associated with older age (r = –0.38, p=0.01), longer disease duration (r = –0.31, p=0.03), more atrophy on volumetric measurement (r = 0.34, p=0.02), more accumulated SLE damage on SLICC scoring (r = –0.36, p=0.01), worse performance on the MMSE test (r = 0.29, p=0.04) and higher HDL cholesterol levels (r = –0.34, p=0.02) (Tables 8.4 and 8.5).

Table 8.4 Univariate relationships between MD and FA with other variables in SLE

	MD			**FA**		
	B	**r**	**p value**	**B**	**r**	**p value**
Age (years)	0.023	0.32	**0.03**	-0.027	-0.38	**0.01**
BMI (kg / m2)	0.024	0.16	0.28	-0.022	-0.15	0.32
Anxiety	-0.022	-0.11	0.45	-0.012	-0.06	0.69
Depression	-0.040	-0.18	0.22	-0.000	-0.00	0.99
Disease duration (months)	0.001	0.23	0.11	-0.002	-0.31	**0.03**
Fatigue	-0.207	-0.35	**0.01**	0.031	0.05	0.72
Brain measures						
FA (n=47)	<0.0001	-0.80	**<0.0001**	NA	NA	NA
MD (n=47) (10-6 mm2 /s)	NA	NA	NA	<0.0001	-0.80	**<0.0001**
ICV (mm3)	<0.0001	-0.12	0.40	<0.0001	0.27	0.06
L hippocampus volume (mm3)	-0.000	-0.19	0.19	0.000	0.16	0.28
R hippocampus volume (mm3)	-0.000	-0.19	0.19	0.000	0.24	0.09
Deep atrophy	0.297	0.31	**0.03**	-0.190	-0.19	0.19
Superficial atrophy	0.389	0.36	**0.01**	-0.281	-0.26	0.07
BTV:ICV	-0.088	-0.38	**0.01**	0.078	0.34	**0.02**
Rheumatology scores						
SLICC	0.292	0.32	**0.03**	-0.325	-0.36	**0.01**
SLEDAI-2K	-0.014	-0.03	0.85	0.004	0.01	0.95
BILAG	-0.025	-0.12	0.40	0.014	0.07	0.63
Cognitive function						
MoCA	-0.109	-0.30	**0.04**	0.042	0.12	0.44
ACER	-0.033	-0.21	0.15	0.025	0.16	0.27
MMSE	-0.178	-0.25	0.08	0.207	0.29	**0.04**
g	-0.348	-0.31	**0.04**	0.261	0.23	0.12
NART	-0.029	-0.27	0.07	0.014	0.13	0.38

Bold p<0.05. ACER = Addenbrooke's Cognitive Examination – Revised, B = beta coefficient, BMI = body mass index, BILAG = British Isles Lupus Assessment Group, BTV = brain tissue volume, g = gneral factor of cognitive function, FA = fractional anisotropy, ICV = intracranial volume, MD = mean difussivity, MoCA = Montreal Cognitive Assessment, MMSE = Mini Mental State Examination, NART = National Adult Reading Test, r = correlation coefficient, SLE = systemic lupus erythematosus, SLEDAI-2K = systemic lupus erythematosus Disease Activity Index 2000, SLICC = Systemic Lupus International Collaborating Clinics

Table 8.5 Univariate relationships between MD and FA with plasma markers in SLE

	MD			FA		
	B	**r**	**p value**	**B**	**r**	**p value**
Rheumatological						
C3 (mg / dL) (n=47)	0.287	0.09	0.53	-0.415	-0.14	0.37
C4 (mg / dL) (n=47)	1.226	0.11	0.48	-1.048	-0.09	0.56
Anti-ds-DNA (IU / mL) (n=47)	-0.004	-0.17	0.25	0.003	0.15	0.32
Inflammatory						
CRP (mg / L) (n=45)	-0.031	-0.24	0.13	0.017	0.13	0.41
ESR (mm / hr) (n=49)	-0.003	-0.05	0.75	0.008	0.13	0.39
IL-6 (pg / mL) (n=40)	-0.133	-0.20	0.23	0.166	0.26	0.12
IFN (RQ value) (n=24)	0.038	0.35	0.11	-0.022	-0.28	0.19
Endothelial dysfunction						
vWF Ag (IU / mL) (n=46)	0.087	0.05	0.73	0.265	0.17	0.27
vWF fVIIIc (IU / mL) (n=46)	0.052	0.02	0.88	0.370	0.17	0.27
vWF RCOF (IU / mL) (n=46)	0.028	0.01	0.95	0.397	0.13	0.39
Homocysteine (umol / L) (n=45)	-0.004	-0.03	0.86	0.002	0.01	0.93
Lipids						
Tot cholesterol (mmol / L) (n=49)	0.218	0.21	0.16	-0.303	-0.29	0.05
HDL cholesterol (mmol / L) (n=47)	0.575	0.23	0.14	-0.833	-0.34	**0.02**
LDL cholesterol (mmol / L) (n=46)	0.207	0.17	0.28	-0.233	-0.19	0.22
Antibodies						
Anti-cardiolipin IgG (GPL) (n=50)	-0.040	-0.13	0.37	0.017	0.06	0.69
Anti-cardiolipin IgM (MPL) (n=50)	0.010	0.08	0.56	-0.015	-0.13	0.39
Lupus anticoagulant (n=47)	-0.104	-0.03	0.83	-0.157	-0.05	0.75

Bold p<0.05. B = beta coefficient, CRP = C-reactive protein, ESR = erythrocyte sedimentation rate, IFN = interferon beta, IL-6 = interleukin-6, r = correlation coefficient, SLE = systemic lupus erythematosus, vWF Ag = von Willebrand factor antigen, vWF fVIIIc = von Willebrand factor VIII, vWF RCOF = von Willebrand factor ristocen co-factor

Fatigue

Fatigue in SLE was significantly higher than the expected normal range[58] (5.0 ± 1.7 *v* 2.3 ± 0.7, p<0.0001). In univariate analyses, higher levels of fatigue were associated with higher depression (*r* = 0.47, p=0.0004), higher anxiety (*r* = 0.41 p=0.002), higher BMI (*r* = 0.36, p=0.01), lower MD (*r* = –0.35, p=0.01), lower IFN (*r* = –0.54, p=0.006) and lower vWF (RCOF (*r* = –0.34, p=0.02) and fVIIIc (*r* = –0.30, p=0.04)) (Tables 8.6 and 8.7). Except vWF (RCOF) all associations remained after adjusting for age (Table 8.8). Except vWF (RCOF) and IFN all associations remained after adjusting for age, disease duration and steroids (Table 8.8).

Table 8.6 Univariate relationships between fatigue (FSS) and cognitive function (*g*) with other variables in SLE patients

	Fatigue			Cognitive function (*g*)		
	B	***r***	**p value**	**B**	***r***	**p value**
Age (years)	-0.028	-0.24	0.09	-0.011	-0.16	0.27
BMI (kg / m^2)	0.093	0.36	**0.01**	-0.025	-0.16	0.25
Anxiety	0.132	0.41	**0.002**	-0.019	-0.10	0.48
Depression	0.174	0.47	**0.0004**	-0.030	-0.13	0.35
Disease duration (months)	0.001	0.10	0.48	-0.003	-0.41	**0.003**
Fatigue	NA	NA	NA	-0.026	-0.04	0.75
Brain measures						
FA (n=47)	-0.088	0.05	0.73	0.202	0.23	0.12
MD (n=47) (10^{-6} mm^2 /s)	-0.580	-0.35	**0.01**	-0.271	-0.31	**0.03**
ICV (mm^3)	0.000	0.19	0.17	-0.000	-0.07	0.61
Left hippocampus volume (mm^3)	0.000	0.04	0.76	0.000	0.28	0.05
Right hippocampus volume (mm^3)	0.000	0.13	0.37	0.000	0.24	0.10
Deep atrophy	-0.217	-0.13	0.35	-0.232	-0.25	0.08
Superficial atrophy	-0.308	-0.16	0.24	-0.274	-0.25	0.07
BTV:ICV	0.059	0.15	0.28	0.051	0.22	0.12
Rheumatology scores						
SLICC	0.016	0.01	0.94	-0.202	-0.22	0.13
SLEDAI-2K	0.091	0.12	0.39	-0.038	-0.08	0.55
BILAG	0.080	0.24	0.09	-0.018	-0.09	0.50
Cognitive function						
MoCA	-0.005	-0.01	0.94	NA	NA	NA
ACER	-0.022	-0.09	0.51	NA	NA	NA
MMSE	-0.002	-0.00	0.99	NA	NA	NA
g	-0.078	-0.04	0.75	NA	NA	NA
NART	-0.002	-0.00	0.95	0.069	0.64	**<0.0001**

Bold p<0.05. ACER = Addenbrooke's Cognitive Examination – Revised, B = beta coefficient, BMI = body mass index, BILAG = British Isles Lupus Assessment Group, BTV = brain tissue volume, *g* = gneral factor of cognition, FA = fractional anisotropy (general factor from 12 tracts), ICV = intracranial volume, MD = mean difussivity (general factor from 12 tracts), MoCA = Montreal Cognitive Assessment, MMSE = Mini Mental State Examination, NART = National Adult Reading Test, *r* = correlation coefficient, SLE = systemic lupus erythematosus, SLEDAI-2K = systemic lupus erythematosus Disease Activity Index 2000, SLICC = Systemic Lupus International Collaborating Clinics

Table 8.7 Univariate relationships between fatigue (FSS) and cognitive function (*g*) with plasma markers in SLE patients

	Fatigue			Cognitive function (*g*)		
	B	***r***	**p value**	**B**	***r***	**p value**
Rheumatological						
C3 (mg / dL) (n=47)	0.680	0.12	0.41	-0.133	-0.04	0.76
C4 (mg / dL) (n=47)	-0.755	-0.04	0.80	-1.331	-0.12	0.41
Anti-ds-DNA (IU / mL) (n=47)	0.002	0.06	0.70	0.003	0.12	0.41
Inflammatory						
CRP (mg / L) (n=45)	0.065	0.29	0.05	0.001	0.00	0.95
ESR (mm / hr) (n=49)	-0.002	-0.02	0.90	-0.007	-0.11	0.43
IL-6 (pg / mL) (n=40)	0.062	0.07	0.66	-0.234	-0.43	**0.006**
IFN (RQ value) (n=24)	-0.091	-0.54	**0.006**	-0.026	-0.33	0.12
Endothelial dysfunction						
vWF Ag (IU / mL) (n=46)	-0.521	-0.20	0.18	-0.799	-0.51	**0.0003**
vWF fVIIIc (IU / mL) (n=46)	-1.168	-0.30	**0.04**	-0.512	-0.22	0.13
vWF RCOF (IU / mL) (n=46)	-1.426	-0.34	**0.02**	-0.889	-0.35	**0.02**
Homocysteine (umol / L) (n=45)	-0.005	-0.02	0.90	-0.025	-0.16	0.29
Lipids						
Tot cholesterol (mmol / L) (n=49)	0.015	0.01	0.95	0.027	0.02	0.86
HDL cholesterol (mmol / L) (n=47)	-0.310	-0.07	0.63	-0.003	-0.00	0.99
LDL cholesterol (mmol / L) (n=46)	-0.194	-0.09	0.54	0.051	0.04	0.78
Antibodies						
Anti-cardiolipin IgG (GPL) (n=50)	0.079	0.15	0.28	-0.000	-0.00	0.99
Anti-cardiolipin IgM (MPL) (n=50)	-0.021	-0.13	0.37	-0.008	-0.08	0.55
Lupus anticoagulant (n=47)	1.297	0.23	0.11	-0.678	-0.19	0.21

Bold p<0.05. B = beta coefficient, CRP = C-reactive protein, ESR = erythrocyte sedimentation rate, *g* = general factor of cognition, IL-6 = interleukin-6, *r* = correlation coefficient, SLE = systemic lupus erythematosus, vWF Ag = von Willebrand factor antigen, vWF fVIIIc = von Willebrand factor VIII, vWF RCOF = von Willebrand factor ristocen co-factor

Cognitive function

One patient was excluded from the analysis of cognitive results as English was not his first language. On average, patients met the minimum levels required for normal cognition by the three individual tests (Table 8.1). However, 19/50 (38%) (MoCA), 13/50 (26%) (ACER) and 5/50 (10%) (MMSE) patients scored below the tests' commonly-used cut-offs indicating clinical levels of cognitive impairment. The mean NART score in SLE was significantly lower than age-matched healthy controls (34 *v* 40, p=0.0008).

In univariate analyses, *poorer* cognitive function (*g*) was associated with longer SLE disease duration (r = –0.41, p=0.003) and higher MD (r = –0.31, p=0.03) (Table 8.6). Poorer cognitive function (*g*) was also associated with higher vWF (Ag (r = –0.51, p=0.0003) and RCOF (r = –0.35, p=0.02)) and higher IL-6 (r = –0.43, p=0.006) (Table 8.7). Except MD, all associations remained after adjusting for age (Table 8.8). After adjusting for age, disease duration, steroids and NART, only higher IL-6 (p=0.02) remained independently and significantly associated with poorer cognitive function (Table 8.8).

Table 8.8 Multiple linear regression models of fatigue and cognitive function in SLE patients

	Unadjusted			Age adjusted			Fully adjusted[a]		
	B	SE B	p value	B	SE B	p value	B	SE B	p value
Fatigue									
BMI (kg / m^2)	0.093	0.034	0.01	0.106	0.033	0.003	0.102	0.033	0.004
Anxiety	0.132	0.041	0.003	0.119	0.044	0.01	0.127	0.044	0.006
Depression	0.175	0.046	0.0004	0.162	0.048	0.001	0.170	0.047	0.001
MD (10^{-6} mm^2 /s)	-0.580	0.234	0.02	-0.556	0.249	0.03	-0.610	0.253	0.02
IFN (RQ value)	-0.090	0.030	0.006	-0.091	0.029	0.005	-0.080	0.040	0.06
vWF (fVIIIc) (IU / mL)	-1.168	0.550	0.04	-1.121	0.539	0.04	-1.111	0.533	0.04
vWF (RCOF) (IU / mL)	-1.426	0.599	0.02	-1.212	0.623	0.06	-1.154	0.616	0.07
Cognitive function									
Disease duration (months)	-0.003	0.001	0.003	-0.003	0.001	0.005	-0.001	0.000	0.07
MD (10^{-6} mm^2 /s)	-0.271	0.126	0.04	-0.264	0.135	0.06	-0.012	0.108	0.91
vWF (Ag) (IU / mL)	-0.800	0.204	0.0003	-0.794	0.215	0.0006	-0.286	0.208	0.18
vWF (RCOF) (IU / mL)	-0.889	0.358	0.02	-0.848	0.380	0.03	-0.296	0.317	0.36
IL-6 (pg / mL)	-0.234	0.080	0.006	-0.241	0.080	0.005	-0.151	0.063	0.02

[a] Fatigue adjusted for age, disease duration and steroids; Cognitive function adusted for age, disease duration, steroids and NART.

B = beta coefficient, IL-6 = interleukin-6, MD = mean difussivity, NART = national adult reading test, SE B = standard error for beta coefficient, vWF Ag = von Willebrand factor antigen, vWF fVIIIc = von Willebrand factor VIII, vWF RCOF = von Willebrand factor ristocen co-factor

Literature review and meta-analysis

The search uncovered 19 papers of which 10 were excluded as not relevant (no measure of MD or FA in SLE or NPSLE). We meta-analysed results in three groups: (1) SLE versus healthy controls, (2) NPSLE versus healthy controls and (3) SLE versus NPSLE.

A total of nine[57,228–235] prior studies were reviewed (n=214 patients; n=24 average per study) and added to the current study for meta-analysis (Table 8.9 and Figures 8.3–8.6). Four studies[229–232] did not provide data to permit inclusion in the forest plots and are summarised with the other studies in Table 8.9 only.

In general, among most tracts, the MD was significantly increased (standardised mean difference (SMD) 1.07 (95% CI, 0.62 to 1.52)) and FA non-significantly reduced (SMD –0.16 (–0.48 to 0.17)) in SLE patients versus healthy controls (Figures 8.3 and 8.4). A similar pattern was seen in NPSLE versus healthy controls. Data comparing SLE to NPSLE directly were limited: only one study[231] provided data on MD, and whereas three studies[228,229,231] reported on FA, there were no data suitable for pooling although the general observation was little difference in water diffusion measures between SLE and NPSLE (Table 8.9).

Table 8.9 Diffusion imaging studies in SLE and NPSLE

Study	Sequence, gradient directions	Field	Technique	n (mean age in years)	Comparison	Measurement	Region	Findings
SLE versus healthy controls								
Zhang, 2007	DTI, 26	1.5T	MTR and ADC histogram, and ROI	34 (47)	SLE v HC	FA and MD	Whole brain and Various regions	↑ MD in brain in SLE ↓ FA in SLE in most regions ↑ MD in SLE in most regions
Emmer, 2010	DTI, 6	3T	TBSS	12 (42)	SLE v HC	FA	Various tracts	↓ FA in SLE in most tracts
Jung, 2010	DTI, 12	1.5T	TBSS	16 (37)	SLE v HC	FA and MD	Various tracts	No difference in FA No difference in MD
Schmidt-Wilcke, 2014	DTI, 15	3T	TBSS & TFCE	19 (38)	SLE v HC	FA	Voxels and clusters across WM	↓ FA in SLE in prefrontal WM
Zimny, 2014	DTI, 25	1.5T	ROI	13 (34)	SLE v HC	FA	14 WM tracts	↓ FA in SLE in most tracts (see FP)
Wiseman, 2015	DTI, 32	1.5T	Quantitative tractography	47 (48)	SLE v HC	FA and MD	8 WM tracts	↓ FA in SLE in most tracts (see FP) ↑ MD in SLE in all tracts (see FP)
NPSLE versus healthy controls								
Bosma, 2003	DWI, 3	1.5T	ADC histogram	11 (35)	NPSLE v HC	mean ADC	Whole brain	↑ mean ADC in brain in NPSLE
Welsh, 2007	DWI, 3	1.5T	ADC histogram	17 (43)	NPSLE v HC	mean ADC	Whole brain and segmented tissues	↑ mean ADC in brain in NPSLE ↑ mean ADC in GM & WM in NPSLE
Hughes, 2007	DTI, 9	1.5T	ROI	8 (43)	NPSLE v HC	FA and MD	Various regions	↓ FA in NPSLE in various regions ↑ MD in NPSLE in various regions
Jung, 2010	DTI, 12	1.5T	TBSS	17 (39)	NPSLE v HC	FA and MD	Various tracts	↓ FA in NPSLE in some tracts ↑ MD in NPSLE in most tracts
Zivadinov, 2013	DTI, 39	3T	Voxel based tissue segment	26 (48)	NPSLE v HC	MD	NAWM	↑ MD in NAWM in NPSLE
Schmidt-Wilcke, 2014	DTI, 15	3T	TBSS	19 (39)	NPSLE v HC	FA	Voxels and clusters across WM	↓ FA in SLE in prefrontal WM
Zimny, 2014	DTI, 25	1.5T	ROI in WM tracts	22 (35)	NPSLE v HC	FA	14 WM tracts	↓ FA in NPSLE in most tracts (see FP)
SLE versus NPSLE								
Jung, 2010	DTI, 12	1.5T	TBSS	16 (37)	SLE v NPSLE	FA and MD	Various tracts	↑ FA in SLE in 2 tracts ↓ MD in SLE in 3 tracts
Schmidt-Wilcke, 2014	DTI, 15	3T	TBSS	19 (38)	SLE v NPSLE	FA	Voxels and clusters across WM	No difference in FA between SLE and NPSLE
Zimny, 2014	DTI, 25	1.5T	ROI	13 (34)	SLE v NPSLE	FA	14 WM tracts	Generally no difference in FA between SLE and NPSLE (see FP)

ADC = average diffusion coefficient, FA = fractional anisotropy, FP = forest plot, GM = grey matter, MD = mean diffusivity, MTR = mean transit ratio, NPSLE = neuropsychiatric SLE, NAT = normal appearing tissue, NAWM = normal appearing white matter, ROI = region of interest, SLE = systemic lupus erythematosus, TBSS = tract based spatial statistics, WM = white matter

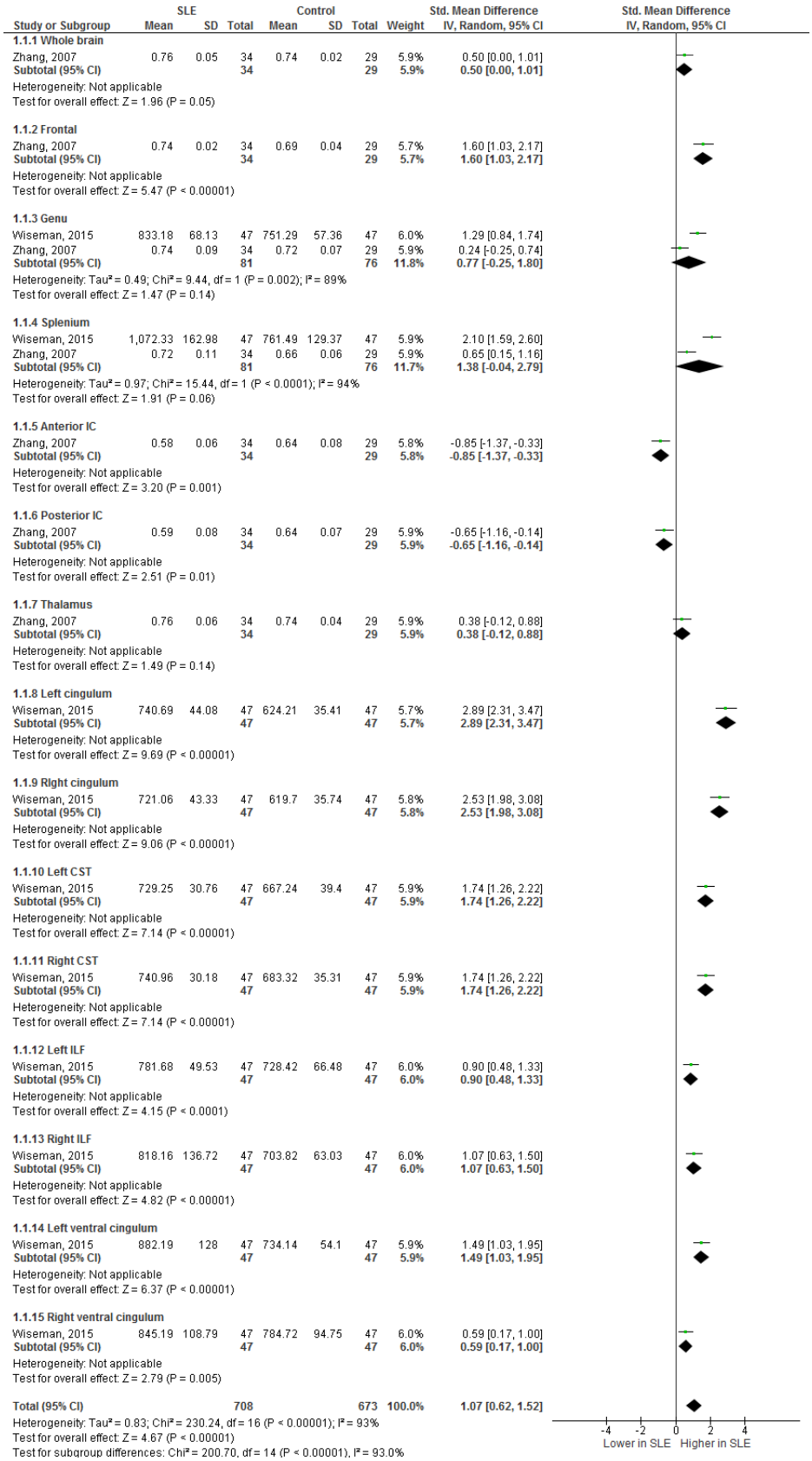


Figure 8.3 SLE versus healthy controls, outcome: mean diffusivity

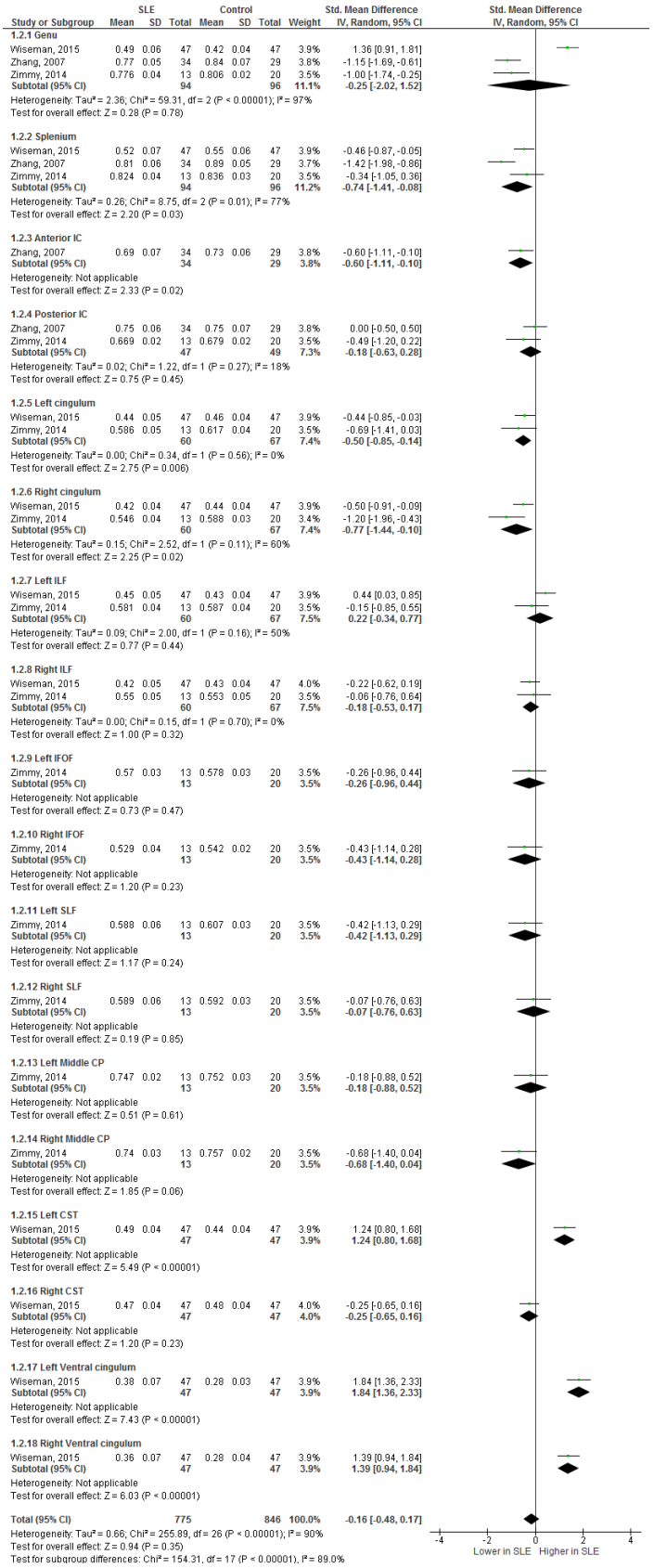


Figure 8.4 SLE versus healthy controls, outcome: fractional anisotropy

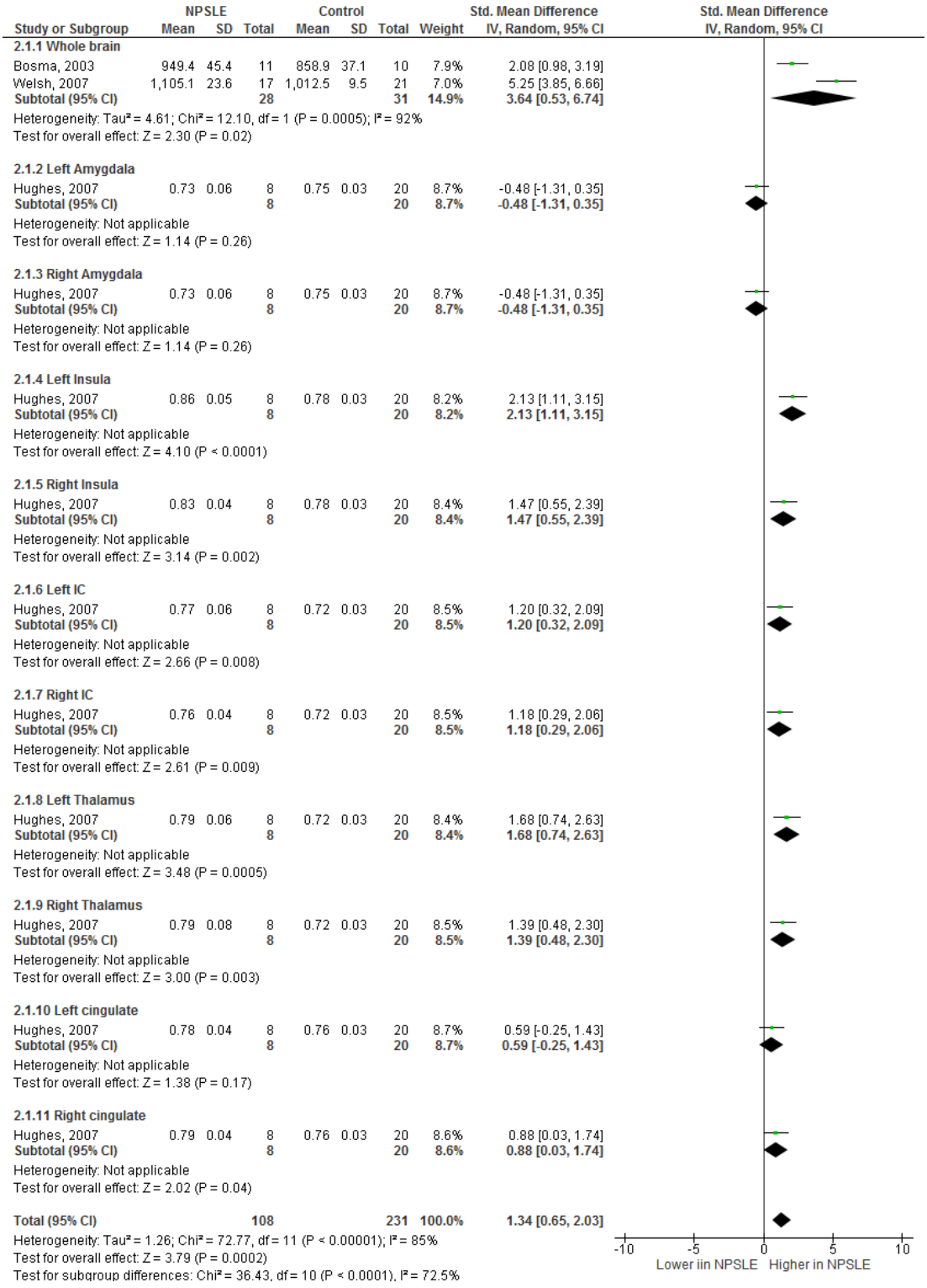


Figure 8.5 NPSLE versus healthy controls, outcome: mean diffusivity

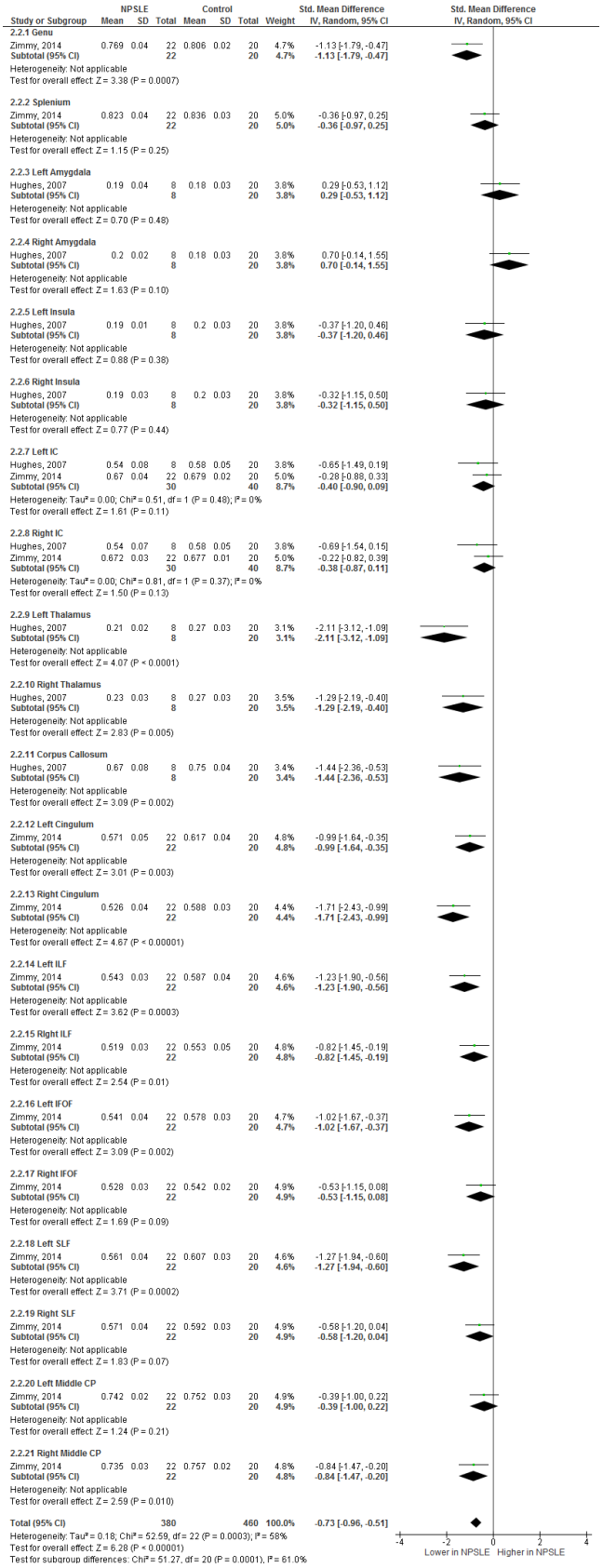


Figure 8.6 NPSLE versus healthy controls, outcome: fractional anisotropy

Discussion

The main findings in the present study, the largest to date on DT-MRI in SLE adding ~25% more data, and the only one to use quantitative tractography, were that: (i) MD was higher in eight major white matter tracts in SLE versus healthy controls; (ii) MD and FA were associated with several expected variables (age, atrophy) but also more permanent damage as measured by SLICC score and lower cognitive function in univariate analyses; (iii) fatigue in patients was higher than a normal reference range and associated with higher anxiety, depression and BMI. Poorer current cognitive function was associated with SLE disease duration and there was an independent association with higher levels of the pro-inflammatory biomarker IL-6.

The direction of the regression slopes relating MD (rising) and FA (declining) with age were as expected but steeper in SLE patients versus controls, indicating accelerated decline in white matter microstructure with age. However, when we tested the slopes using an interaction term in a linear regression model there was no significant difference between patients and controls and a larger sample will be needed to confirm accelerated ageing. Nonetheless, significantly higher MD levels were found in all white matter tracts in SLE patients versus healthy controls which are in accordance with the literature review of nine prior studies that used diffusion imaging. This indicates a diffuse increase in brain water mobility in SLE, possibly indicating a subtle decline in white matter microstructural integrity. We recently demonstrated increased stroke risk in SLE versus the general population with greatest risk in those <50 years; again possibly indicative of accelerated ageing[56] (see Chapter 4) and the results here are also consistent with our findings in Chapter 7 of more markers of SVD in SLE patients versus healthy controls.

In SLE, higher fatigue was also associated with lower MD, although this likely reflects higher fatigue scores being more common in younger participants. A much larger study will be required to determine if fatigue in SLE is associated with reduced MD levels for a given age. Higher fatigue was also associated with lower IFN, lower vWF (fVIIIc and RCOF) and approached significance with higher CRP.

The finding that higher fatigue was associated with lower MD was unexpected, although the most fatigued were also the youngest which partly explains our finding as younger people have lower MD. No other studies have examined MD with fatigue in SLE. In a prior study of ankylosing spondylitis patients with high fatigue (n=20; method = Tract-based Spatial Statistics (TBSS)) there was reduced FA values in several tracts (inferior fronto-occipital fasciculi, superior/inferior longitudinal fasciculi and corticothalamic tracts) versus healthy controls.[277] Conversely, patients diagnosed with granulomatosis with polyangiitis (n=28; method = TBSS) were dichotomised into 14 with and 14 without fatigue: fatigued patients had *increased* FA in the let fornix and bilateral posterior cingulum bundles which the authors suggest may reflect plasticity secondary to normal activation of these tracts.[278]

A possible explanation of our fatigue-MD finding apart from age was steroid use. We have previously shown steroids reduce MD values in oedematous brain acutely in patients with brain tumours[279]. Corticosteroids are commonly prescribed in the treatment of SLE, although we did not quantify current/lifetime dose and so could not directly relate our DT-MRI findings to steroid dose as a continuous variable. However, fatigue remained inversely associated with MD in the multivariable model that included age and current steroids as a dichotomised variable.

Higher levels of fatigue were associated with higher BMI in the current study, as has been established previously[280]. Higher levels of fatigue were also associated with lower levels of endothelial dysfunction (vWF). The plasma marker that had the highest association with fatigue was IFN, independent of age. Fatigue is a common side effect of IFN therapy although none of the current SLE study participants were on IFN thereapy; meanwhile, prior studies of endogenous IFN in SLE did not show associations with fatigue.[281,282]

Lower levels of current cognitive function were associated with longer SLE disease duration and higher MD and between 10% and 38% of patients had cognitive test scores indicating clinical levels of cognitive impairment. Our finding that lower levels of cognitive function were associated with higher MD is in agreement with Bosma et al.[226] who examined 24 patients diagnosed with NPSLE, although when we adjusted for age the association disappeared (as people get older MD tends to rise). Bosma and colleagues also correlated lower levels of neurological functioning (essentially motor skills) with higher levels of magnetization transfer MRI parameters.

Poorer current cognitive function was also associated with higher levels of the pro-inflammatory cytokine IL-6, independent of age and prior cognitive abilities. We used a high-sensitivity enzyme-linked immunosorbent assay (R&D Systems, Abingdon, UK) with sensitivity of 0.16 pg/mL. The PROSPER[283] study (randomised controlled trial; n=5,653) associated higher IL-6 with worse executive function ($p<0.001$), independent of age. At follow-up (mean 39 months), higher IL-6 was independently associated with an increased rate of cognitive decline in both executive function

(p=0.002) and memory (p=0.002)[283]. The mean age of participants in the PROSPER study was 75 years, considerably older than the mean age of participants in the current study, yet the similarity in findings could indicate that SLE patients are experiencing aged-related effects on the brain at younger ages. The PROSPER study corrected for educational level but not premorbid intelligence using NART.

Other variables associated with cognitive function on univariate analysis became nonsignificant when NART was added to the regression models. The apparent current cognitive impairment is thus mostly explained by premorbid IQ, although inflammation also played a role in this data.

Strengths of the present study include use of a continuum of fatigue and cognitive scores rather than dichotomised data, the 32 diffusion-encoding gradient directions in the DT-MRI exam which increases the precision of the imaging data, a large sample size relative to existing DT-MRI studies (although we note our sample is still small, limiting study power) in SLE and use of quantitative tractography rather than 'region of interest' or 'voxel-based' methods. The quantitative tractography method employed here, PNT, has the advantage that it segments white matter tracts automatically in native space, avoiding brain distortion by use of registration to standard space and thereby providing objective measures of tract microstructure that can be correlated with phenotypic data. Additionally, our study did not solely focus on patients that were neurologically symptomatic or diagnosed with NPSLE but instead included a range of SLE patients making our findings relevant to the wider SLE patient population. In the multiple linear regression models we corrected for age, disease duration, and where current cognitive function was the outcome (dependent variable), NART, to adjust for an inferred prior (peak) IQ.

We did not administer tasks to assess reaction times, information processing speed or motor skills and so we are unable to comment on these aspects of neurological function. Although subjects were asked to consider their fatigue over the prior week, they were seen at different times of the day and this could have impacted the self-reported fatigue scores via diurnal variation.

The main observation from the literature review and meta-analysis (increased MD and decreased FA in SLE versus controls) was in agreement with findings in this study. Studies of other inflammatory autoimmune diseases show a similar pattern of findings, for example, in Sjögren Syndrome (n=19; method = TBSS) there was increased MD and decreased FA in several tracts compared to controls[284].

Conclusion

Patients with SLE have more microstructural brain white matter damage for age than the general population, but this does not explain increased fatigue or lower cognition in SLE. Lower MD in the most fatigued may reflect younger age which should be explored in larger studies. Fatigue associates with BMI, anxiety and depression.

Worse current cognitive function in SLE is related to lower prior cognitive ability although inflammation also plays a detrimental role and the independent association with raised IL-6 should be explored in larger datasets.

The findings are consistent with the increased SVD burden shown in the previous chapter and adds evidence of microstructural brain damage to overt SVD features in SLE. This likely contributes to the 'brain fog' described by most SLE patients. Larger studies are needed and these are clearly feasible based on this pilot study, as has been described in concluding paragraph to Chapter 7.

Chapter 9: General discussion

This aims of this thesis were to:

- investigate plasma biomarkers of coagulation, fibrinolysis, endothelial dysfunction, and inflammation in lacunar stroke versus other ischaemic stroke subtypes and non-stroke controls;
- assess relationships between plasma biomarkers and SVD features such as WMH;
- review associations between stroke, including stroke subtypes, and rheumatic diseases, and to determine if rheumatic diseases increase the risk of specific stroke subtypes or 'silent' vascular disease on neuroimaging; and,
- via the conduct of a pilot MRI neuroimaging study:
    - assess the burden of SVD among patients with the inflammatory disease SLE versus a cohort of stroke patients (i.e., clinically overt SVD) and non-stroke healthy controls;
    - assess relationships between total SVD burden and plasma biomarkers of SLE disease activity, inflammation, endothelial dysfunction, cholesterol and autoantibodies;
    - investigate evidence of SVD burden in the brain including microstructural damage in SLE including relationships with cognition, fatigue, SLE disease activity and inflammatory markers.

We have shown an increase in SVD burden in the inflammatory rheumatic disease SLE (Chapter 7) and an association with stroke at younger ages in RA, SLE and other inflammatory rheumatic diseases (Chapter 4) which has been unexplained to date. The increased SVD burden, seen mainly as enlarged PVS on scanning, is consistent with

perivascular inflammation and provides mechanistic evidence that systemic inflammation may affect endothelial function and lead to brain damage. The aims and main findings on a chapter-by-chapter basis are summarised below, followed by a general discussion, including comments on the difficulties encountered and implications for future research.

In Chapter 2, we systematically reviewed the literature for studies comparing plasma biomarkers of coagulation, fibrinolysis, endothelial dysfunction and inflammation in lacunar stroke versus non-stroke controls or other ischemic stroke subtypes. Markers of ***coagulation/fibrinolysis*** (t-PA, PAI, fibrinogen, D-dimer) were higher in lacunar stroke patients versus non-stroke controls. There was no difference in the levels of t-PA and PAI between lacunar stroke and non-lacunar stroke. Fibrinogen and D-dimer were significantly lower in lacunar stroke compared to other ischemic stroke subtypes. Markers of ***endothelial dysfunction*** (homocysteine, vWF, E-selectin, P-selectin, ICAM, VCAM) were higher or had insufficient or conflicting data in lacunar stroke versus non-stroke. Compared to other ischemic stroke subtypes, homocysteine did not differ in lacunar stroke, while vWF was significantly lower in lacunar stroke. Available data did not permit meta-analysis of E-selectin, P-selectin, ICAM and VCAM but the general impression was of no difference in these biomarkers between stroke subtypes. Markers of ***inflammation*** (CRP, TNF-α, IL-6) were higher in lacunar stroke versus non-stroke. Compared to other ischemic stroke subtypes, there was no difference (CRP) or insufficient or conflicting data (TNF-α) to lacunar stroke. IL-6 was significantly lower in lacunar stroke versus other ischaemic stroke subtypes. We concluded that more studies comparing lacunar stroke to non-lacunar stroke specifically, rather than to non-stroke controls, are needed. The available data in the

review were limited and did not exclude the possibility that peripheral inflammatory processes including endothelial dysfunction are associated with lacunar stroke and SVD[127].

In Chapter 3, we used data from a prospective study of patients presenting with non-disabling lacunar or cortical ischaemic stroke. Biomarkers of inflammation, endothelial dysfunction and haemostasis were measured and compared between the two stroke groups. We calculated WMH volume and modelled it as a function of age, sex, hypertension and smoking (the baseline model). We fitted exploratory models using plasma biomarkers as additional predictor variables to assess model improvement over baseline. The lacunar group had lower t-PA levels in adjusted analyses compared with the cortical group. There were no significant differences in the other plasma biomarkers. The direction of the t-PA finding was consistent with our updated meta-analysis (new total 300 lacunar strokes), although the overall effect remained non-significant. The baseline regression model explained 29% of the variance in WMH volume. Inflammatory biomarkers showed minor improvement over the baseline model, but the other plasma biomarkers did not improve the baseline model. We concluded that plasma t-PA levels appear to differ between lacunar and cortical stroke subtypes, independent of age, sex and vascular risk factors and may reflect endothelial dysfunction, but found no difference in the other plasma biomarkers. Except for a minor additional predictive effect of inflammatory markers, plasma biomarkers did not relate to WMH severity in this small stroke population[285].

In Chapter 4, we reviewed associations between stroke and rheumatic disease, including incidence rates and pooled rate ratios for stroke subtypes versus the general population. We also assessed risk by age and determined if rheumatic diseases

increased the risk of 'silent' vascular disease on neuroimaging. Prior published meta-analyses and new pooled analyses of any stroke in RA, SLE, ankylosing spondylitis, gout and psoriasis showed an excess risk of stroke over the general population. New meta-analyses of stroke subtypes in RA and SLE also showed an excess risk of stroke over the general population. Stroke risk across rheumatic diseases was highest in those aged <50 years and reduced relatively with ageing as the general population caught up with the increased risk. We concluded risk of any stroke is higher in most rheumatic diseases than in the general population, particularly in patients aged <50 years.

In Chapter 5, we used data from an audit of a large regional rheumatology service which we linked to Scottish national hospital records to assess the number of strokes, including subtypes, across several common rheumatic diseases. We grouped the arthropathies into inflammatory and degenerative (non-inflammatory) categories to see if there was a higher burden of all stroke or of specific stroke subtypes among the inflammatory group, investigated age at stroke to confirm if stroke in inflammatory versus degenerative arthropathies occurred at a younger age in these patients, and set our findings in context by comparing to stroke incidence rates and age at stroke from the general UK population. There were 347 cerebrovascular disease event episodes among 224/6,613 (3.4%) patients. We classified 4,088/6,613 (62%) rheumatology patients as inflammatory and 664/6,613 (10%) as non-inflammatory. Of these, 157/4,088 (3.8%) and 28/664 (4.2%) had cerebrovascular events. Inflammatory arthritis was not associated with cerebrovascular events before and after adjusting for age and sex. Age was predictive of cerebrovascular events: a year's increase in age resulted in a 6% increase in likelihood of having an event. The mean age at stroke event in the rheumatic disease population overall was 63.7 ± 12.9 years with a peak in

events in the 55–64 years age band, which is two decades earlier than in the UK general population. We concluded that there was no difference in the *proportion* of strokes occurring between patients classified as inflammatory versus non-inflammatory, but that strokes in rheumatic diseases occur at younger ages than is normally expected, consistent with our finding of accelerated stroke risk in rheumatic patients in Chapter 4.

In Chapter 6 we described the background and rationale for pilot MRI neuroimaging study of patients diagnosed with the inflammatory autoimmune disease SLE, and in Chapters 7 and 8 gave the results to the study. We hypothesized that patients diagnosed with SLE would have evidence of SVD on brain MRI, and this could be the biological basis for fatigue and cognitive decline, and might explain increased stroke risk. We sought to provide evidence that systemic inflammation can be associated with cerebral perivascular inflammation in SVD.

Of 55 consecutive patients with SLE invited to participate, 51 (93%) agreed and were compared with 51 healthy controls and 51 stroke patients. Compared to healthy controls, SLE patients had a greater total SVD score sustained across each 10-year age band, including more deep but not periventricular WMHs. Compared to stroke patients, the SLE patients also had higher SVD score, mostly due to having more PVS. The total SVD score was not associated with SLE activity, accumulated systemic damage, nor SLE disease duration in this small sample. We concluded that patients with SLE have more SVD markers, notably PVS and deep WMH, than sex- and age-matched healthy controls and mild stroke patients from the same health region.

In tractography analyses, MD was significantly higher in all tracts in SLE patients versus controls, which was consistent with our meta-analysis of the literature (n=10 studies; n=261 SLE patients). We concluded that patients with SLE have more microstructural brain white matter damage for age than the general population which may explain the consistently (though subtly) altered cognitive function, but does not explain increased fatigue in SLE. The findings of more microstructural damage was consistent with the increased SVD burden, and added evidence of microstructural brain damage to overt SVD features in SLE. This may contribute to the 'brain fog' described by most SLE patients and also recognised (anecdotally) to be a complaint amongst older patients with sporadic SVD. We show that a larger study would be very feasible based on our pilot study.

General discussion – vascular / atherothrombotic

Stroke is a preventable and treatable disease[286]. Recent advances such as better public awareness that stroke is a medical emergency and the signs to look for, use of aspirin, thrombolysis, multidisciplinary rehabilitation and specialised acute stroke units have improved outcomes for patients, but the burden of stroke is still high, probably because society is ageing and people – at the population level – take a laissez-faire attitude to altering diet and sedentary habits, at least until – at the individual level – a warning sign is delivered. Dietary choices influence stroke risk. Eating more fruit and vegetables confers protection from stroke in general[287]. Plant-based foods are also high in fibre[7,8] and potassium[288] which have been associated with lowering stroke risk by up to a fifth.

The conventional risk factors for stroke (hypertension, smoking, hyperlipidemia, diabetes, hyperhomocysteinemia) are modifiable in those willing, able and dedicated enough to take control of their heath, although some risk factors are unavoidable (increasing age) and there are a proportion of people that have systemic inflammatory diseases (e.g. RA and SLE) that increase stroke risk and accelerate atherosclerosis, although here too lifestyle choices may attenuate risk by dampening the inflammation.

The conventional risk factors for stroke do not fully account for stroke incidence, and some people that have had a stroke do not have any of these risk factors and so alternative mechanisms need to be considered. Such alternative mechanisms might act alone in some strokes, but in most cases the likely mode of operation is interaction with the traditional stroke risk factors to precipitate or accelerate stroke.

Atherosclerosis is now known to be a chronic inflammatory disease[44,289] involving innate and adaptive immunity (macrophages, T cells and mast cells)[44,217,289–291], triggered by high levels of circulating cholesterol, which also accumulates in the arterial wall. In the smaller vessels there is intimal thickening[217]. The fat trapped in the vascular system (why this happens is not known, but is probably one mechanism the body uses to neutralise too much circulating cholesterol) becomes oxidised and phagocytosed by macrophages leading to fat-laden macrophages (foam cells). Lipid oxidation upregulates adhesion molecules and promotes transmigration of immune cells from the circulation and into the vascular wall. Subendothelial accumulation of lipids (fatty streaks) and foam cells develop a protective fibrous ‘cap’ which can be infiltrated by activated T cells, which produce pro-inflammatory mediators and enzymes, and, in cases of plaque rupture, thrombosis – which is followed by platelet activation and aggregation, and coagulation.

While the accumulation of lipids in the arterial wall is considered a long term phenomenon that develops over time, even a single meal[292] high in animal fat has been linked to an immediate vascular stiffening, inhibiting normal vasodilation, and has also been found in Asian males[293]. The endotoxins present in animal fat and protein survive cooking, stomach acid and enzyme digestion, and trigger an inflammatory response[294]. In mice, a systemic challenge with the bacterial endotoxin lipopolysaccharide exacerbates ischaemic brain damage and the severity of neurological deficit, while simultaneous administration of interleukin-1 receptor antagonist attenuated the effect[38]. Obese mice have worse outcomes[295] and raised plasma concentrations of the pro-inflammatory cytokine IL-6[296] after stroke compared to lean controls, highlighting the interaction of the peripheral inflammatory response on the brain.

General discussion – SVD

However, questions remain unanswered as to whether inflammation outside the brain plays a role in the microvascular damage that is well-documented in lacunar stroke or the development or worsening of SVD. Systemic inflammation might predispose to a higher burden of SVD, possibly by increasing vascular endothelial injury in the perforating arterioles. Indeed, this thesis has shown evidence of the association of systemic inflammation and SVD, for instance via the increased burden of enlarged PVS in SLE patients. However, the only association we found with elevated markers of inflammation in lacunar stroke specifically was when lacunar stroke was compared to non-stroke healthy controls (raised levels of all of CRP, IL-6 and TNF-α; Chapter 2) and we did not find an association between raised plasma inflammatory markers and lacunar stroke versus other ischaemic strokes in the systematic review[127] or our study of two types of mild ischaemic stroke patients[285]. This suggests that

inflammatory plasma marker elevation in lacunar stroke is likely to reflect the process of having a stroke, rather than that systemic inflammation or endothelial dysfunction following stroke is specific to lacunar stroke alone, although we cannot exclude the possibility that systemic inflammation *prior to* first ever stroke is specific to lacunar stroke. Also, we cannot exclude the possibility that a raised inflammatory profile increases the risk of any stroke and that the subtype of stroke is determined more by other characteristics than the inflammation per se.

The Levels of Inflammatory Markers in the Treatment of Stroke (LIMITS) study was nested within SPS3[297], a multi-centre secondary prevention trial in stroke patients with small vessel stroke. Among a number of inflammatory markers, the LIMITS investigators measured the inflammatory biomarkers CRP[298] and IL-6[299] a minimum of three weeks after stroke in 1,244 lacunar stroke patients and again about one year later. CRP and IL-6 levels predicted the risk of recurrent stroke and other major vascular events, although the study did not have sufficient power to predict recurrent small vessel stroke specifically, and had no non-lacunar stroke control group. Whether inflammatory markers are causative of recurrent stroke, or merely co-associated modulators, needs to be better understood.

The LIMITS study may be confounded because they studied patients that already had a stroke – and so the inflammatory profile will be raised because of the stroke, even though the LIMITS investigators attempted to control for this by drawing blood at least three weeks post-stroke in an effort to allow the acute inflammatory response to settle. Most plasma biomarker studies draw blood in the acute phase following stroke which is confounding because the immune response is active resulting in raised circulating markers of inflammation. Between-study heterogeneity on time to blood draw is an

issue in biomarker studies, but we dealt with this in our meta-analysis (Chapter 2) by grouping studies into acute and chronic, using 21 days as the cut point. In our study of mild ischaemic stroke subtypes (Chapter 3) we drew blood a minimum of one month after the strokes.

Many imaging features of SVD that are detected on MRI, especially in older people, do not produce clinical symptoms, yet non-stroke 'silent' SVD is important because these SVD imaging features are associated with an increased risk of stroke and cognitive decline. In a large cross-sectional study of neurologically normal non-stroke patients (n=519), Mitaki et al.[49] showed CRP was associated with [silent] lacunar infarcts (n=54, p=0.02), microbleeds (n=26, p=0.03) and more severe deep (n=114, p=0.04) and periventricular WMH (n=43, p=0.04). However, the authors used tertiles of CRP, and these associations reflected the comparison of the lowest tertile of CRP against the highest, which throws away the middle third of the data. The authors then modelled presence or absence of each of the imaging feature of SVD (as outcome variables) using binary logistic regression with CRP as a continuous predictor variable (a much better approach as it uses the entire continuum of data) and found higher levels of CRP were associated with silent lacunar infracts after adjusting for age, sex, hypertension, diabetes, hyperlipidemia, smoking and alcohol (odds ratio 1.62, 95% CI 1.12–2.33, p=0.009); the other imaging markers of SVD were not related to levels of CRP. The authors did not control for pre-existing inflammatory diseases, and the sample size (n=54 lacunar infarcts) is probably too small to be generalizable. Wersching et al.[129] also found no association between CRP and WMH among 321 stroke-free participants, although higher levels of CRP were associated with worse performance on cognitive testing after adjustment for age, education and

cardiovascular risk factors (ß = –0.095, p=0.02) and reduced FA (ß = –0.237, p<0.001) indicating cerebral microstructural damage in the absence of an association with inflammation. Aribisala et al.[48] found no association between inflammation (a latent factor comprising CRP, fibrinogen and IL-6) and WMH among 634 community-dwelling older people of near-identical age, after adjustment for risk factors, although did show an association between inflammatory markers and increased PVS, and in turn, between increased PVS and increased WMH. Shoamanesh et al.[131] found no association between inflammatory biomarkers (CRP, IL-6 and TNF-α) and silent infarcts or extensive WMH in the stroke-free Framingham cohort (n=522) but did associate ICAM (a marker of endothelial activation) with these SVD features.

In contrast, three large population studies of non-stroke subjects (n=1,841 in the 3C Dijon Study[67], n=1,033 in the Rotterdam Scan Study[128] and n=3,437 in the Cardiovascular Health Study[300]) found higher inflammatory biomarkers were independently associated with higher WMH, although the 3C-Dijon Study used an automated imaging processing technique to calculate WMH volume which might have miscalculated the volume of WMH by including scan artefacts while the Cardiovascular Health Study did not appear to use a validated WMH rating scale.

These differing findings urgently need meta-analysis to get a more complete view of the association, or not, of plasma biomarkers of inflammation in silent SVD to augment our review of plasma biomarkers in clinically relevant SVD[127] with consideration of the methodological strength of the underlying study designs.

Inflammation, including systemic non-resolving inflammation, goes though periods of active flare and quiescence, which could explain why some studies find associations

and others do not. Alternatively, differing inflammatory pathways may underlie SVD. A promising line of enquiring is endothelial dysfunction and perivascular inflammation. Biomarkers of endothelial activation were associated with WMH in cross sectional analysis[132] and with WMH progression[69]. In the Framingham Heart Study[131], ICAM was associated with silent infarcts and WMH after adjusting for age (n=522, odds ratio 1.7, 95% CI 1.1–2.5, p=0.02). Flow-mediated dilatation studies have shown endothelial dysfunction in lacunar stroke versus non-stroke controls[133]. Biomarkers of endothelial dysfunction are associated with greater arterial stiffness in longitudinal studies[301]. Perivascular inflammation of the small cerebral vessels is a prominent finding in SLE[267] at autopsy as well as in sporadic SVD[46]. Some studies have noted PVS[208,268] on brain imaging in SLE patients (n=122). Endothelial dysfunction is also implicated in SVD[150]. After adjusting for age, sex and vascular risk factors, Aribisala et al.[48] found inflammation was associated with PVS (ß=0.12, p=0.048). In an interventional study[302], there was a significant boost in arterial function in coronary artery disease patients placed on a low fat, plant-based diet for 12 weeks versus age- and risk-factor matched controls. In a double-blind randomized controlled trial, vitamin B supplementation (needed to detoxify homocysteine) slowed the rate of brain atrophy in people with mild cognitive impairment[303] although the study only provides weak evidence as it was small (n=168) and lost 25% of participants to follow-up; a larger trial is required to confirm findings. Petri et al.[220] found homocysteine, a marker of endothelial dysfunction, to be an independent risk factor for stroke in SLE while Jeon et al.[304] found an association between homocysteine and ischaemic stroke (n=825), including small vessel stroke, after adjustment for age, sex, hypertension, diabetes, smoking and alcohol consumption but not meat consumption

(odds ratio 1.04, 95% CI 1.01–1.07, p=0.005). In our SLE study (Chapters 7 and 8) we show that 37/45 (82%) patients with SLE had elevated homocysteine.

Comorbid inflammatory diseases

None of the lacunar stroke and 'silent' SVD studies adjusted for comorbid inflammatory diseases. Studying patients with chronic inflammatory diseases (at the cellular and epidemiological level) could provide useful mechanistic insights into lacunar stroke and SVD because the inflammation in these patients is active and, like stroke in general, probably interacts with traditional vascular risk factors to accelerate SVD. Similarities between atherosclerosis and chronic inflammatory rheumatic diseases are seen, such as activation of macrophages and increased levels of circulating cytokines[305,306]. The reason for early entry of immune cells into the perivascular tissue is not clear but the resultant release of inflammatory mediators can make the endothelium adherent and procoagulant (e.g. upregulation of tissue factor and PAI-1 and downregulation of t-PA)[217,307,308]. In Chapter 3 we saw lower levels of t-PA in lacunar stroke patients versus cortical stroke patients, although in meta-analyses (Chapter 2) the overall effect showed no difference in t-PA levels between these two ischaemic stroke subtypes even though the directionality of our finding was consistent. Knottnerus et al.[150] found higher levels of t-PA in SVD patients that had extensive WMH versus those with an isolated lacunar infarct, suggesting differences in the levels of t-PA at different stages of SVD, or different mechanisms between these lacunar stroke subtypes, although there was no association between extensive WMH and t-PA levels when age was adjusted for. Increased endothelial disturbance results in increased t-PA secretion but also increased levels of its inhibitor, tissue plasminogen

activator inhibitor (PAI), resulting in increased levels of circulating complexes with t-PA[143–145] which complicates, and potentially confounds, analyses. The largest study to date to find lower levels of t-PA in small vessel stroke is the Sahlgrenska cohort, Sweden[92]: among 600 patients with ischaemic stroke, including 124 with small vessel stroke, small vessel stroke patients had higher t-PA levels compared to non-stroke controls in the acute phase and at three months, but lower t-PA levels compared to other stroke subtypes which was consistent with our Chapter 3 findings[285].

Clearly, an over-active or non-resolving inflammatory immune response can disrupt the vascular system, including the small vessels. Epidemiology studies have shown a risk reduction of cardiovascular disease when the pro-inflammatory cytokine TNF-α is blocked[309]. Clinical trials of inflammation modification in atheromatous vascular disease are ongoing. Methotrexate is a very common disease-modifying anti-rheumatic and anti-inflammatory drug which has been used in the treatment of inflammatory rheumatic diseases such as RA and SLE for several years. The CIRT (Cardiovascular Inflammation Reduction Trial) trial is an ongoing, randomised, double-blind, placebo-controlled trial of low dose methotrexate to see if it reduces heart attack, stroke or death in people with type II diabetes or metabolic syndrome that have had a heart attack. This would be targeting large artery atheroma mainly. The investigators are hoping to enrol 7,000 patients over the period 2013 to 2018.

Atrial fibrillation (AF) contributes to ischaemic stroke. Anticoagulation reduces stroke in AF patients, but not completely, and some treated patients still go on to have a stroke. Colchicine is an anti-inflammatory drug used in the treatment of gout. The CIAFS-1 (Colchicine in Atrial Fibrillation to Prevent Stroke) trial is a much smaller (enrolment target, n=60) ongoing, randomised, placebo-controlled trial of colchicine

to see if it reduces stroke in anticoagulated patients with AF. Colchicine is also being tested in the CONVINCE (COlchicine for preventioN of Vascular Inflammation in Non-CardioEmbolic stroke) trial, a randomised clinical trial of low-dose colchicine for secondary prevention after stroke (EU Clinical Trials Register 2015-004505-16). Colchicine has shown efficacy in preventing coronary events when used with statins versus statins alone[310]. Moreover, others[289,311] suggest further studies are needed to evaluate the use of immune-directed therapies in atherosclerotic cardiovascular disease, including use of novel approaches to monitor inflammation at the site of disease (e.g., with 18F-fluorodeoxyglycose positron emission tomography or fluorine-19 MRI), a sentiment that could be extended to cerebral SVD.

In our pilot neuroimaging study, we showed that patients with the inflammatory autoimmune disease SLE had a higher burden of SVD, notably more enlarged PVS. Enlarged PVS were associated with inflammation (n=634) in prior work[48] at our Centre. We also meta-analysed the literature on stroke risk in SLE patients which showed a doubling of risk over the general population, although the data on lacunar stroke specifically were limited and insufficient for meta-analysis. Clearly, patients with inflammatory rheumatic diseases exhibit a pro-inflammatory phenotype and are at increased stroke risk which may in part be mediated by accelerated microscopic damage as seen by increased SVD burden. The peripheral inflammation in inflammatory rheumatic diseases could modulate stroke vulnerability via endothelial dysfunction or increase susceptibility to coagulability as well as lead to damage secondary to perivascular inflammation. Stroke risk is also increased in other inflammatory diseases such as inflammatory bowel disease[312,313] and SVD imaging features have been reported[314,315] including microstructural damage derived from DT-

MRI indices of water molecule diffusivity[316]. Additionally, we showed that stroke risk in rheumatic diseases occurs earlier in life (in our meta-analysis in Chapter 4 and the national linkage search in Chapter 5), supporting the hypothesis that active inflammation promotes accelerated cerebrovascular disease including microvascular disease.

Difficulties

In Chapter 2 we conducted a systematic review and meta-analysis of 13 plasma biomarkers of four physiological processes in lacunar stroke versus non-lacunar stroke (to control for having any stroke) and non-stroke (to compare to the general population) and in Chapter 3 we compared eight biomarkers between lacunar and cortical stroke obtained from a stroke study conducted at our Centre[134]. Choosing biomarkers is not straightforward. The ones studied might be benign while valuable information might go undetected in those not studied. There are also financial and technical limitations, for instance we did not study markers in CSF as CSF is rarely obtained in these patients. We based our choices on prior literature, biological plausibility and advice from experts in inflammation and plasma markers. We cannot exclude the possibility of missing associations because of our choice of biomarkers. Conversely, biomarkers that measure the same biological process tend to be correlated (e.g., IL-6 correlates with TNF-α) and so one might infer that if inflammation (measured by IL-6) correlates with a brain imaging feature of SVD, then TNF-α (or some other inflammatory marker not studied) probably does too via co-association. Sample size is often a problem – most biomarker studies are not large enough to find subtle but important associations. Lastly, as the study in Chapter 3 was cross-sectional we are unable to comment on causation.

In Chapter 4 we reviewed cerebrovascular disease in rheumatic disease. We focussed our analysis on the main rheumatic diseases – in order to capture the general theme – yet there are several more rheumatic diseases that could have been studied as well as non-rheumatoid inflammatory diseases such as Crohn's that could have augmented the analysis, although arguably such diseases have sufficiently different pathogenic processes that they warrant their own review. These topics could be addressed in future. Additionally, it would be interesting to know if the aggressive pharmaceutical management of those with the most severe inflammation attenuated stroke risk or halted (or increased) the development of WMHs and other biomarkers of SVD, although extracting this level of detail from the literature could be challenging as it is generally not reported. Data on ischaemic stroke subtypes was limited, and so we were only able to provide an estimate of lacunar stroke incidence rate and not pooled risk versus the general population (most studies do not subtype stroke and those that do use varying methods; this is a known problem). A large brain imaging population study with detailed stroke phenotyping is needed to fully characterise stroke subtypes including SVD in rheumatic patients but would require a large sample and long term follow up to achieve reasonable power.

In Chapter 5 we conducted a national data linkage study of rheumatology patients in NHS Lothian, with cerebrovascular disease events as the outcome measure. The input data involved 6,613 patients but a third of the data could not be used as the diagnoses were incomplete. We attempted to correct this by re-analysing the audit records for information that would allow us to classify the missing data (for example a single patient's data entry might show no clear diagnosis but the notes field might say "probable RA" and we could have legitimately allocated this patient to the RA group)

but the scale of this task became insurmountable in the timeframe of this thesis. Work is ongoing to rectify this problem so that the sample size, hence study power, can be increased before re-analysing the data. A future study would aim to allocate diagnosis at inception as closely as possible and follow-up to confirm or exclude each patient.

In Chapters 6, 7 and 8 we report on our SLE study. We encountered relatively few problems with this study. DT-MRI failed in four patients which reduced our sample size from 51 to 47 for the tractography measurements. We considered re-calling these four subjects but resource limitations precluded this, and it was a pilot study. We used quick screening tools rather than a full psychological battery in our assessment of cognitive function in the SLE subjects but we justified this on the basis that this was a pilot project. The tests were chosen for their ease of use, practicality, validity, wide evidence base and relevance to medical care and patients. Therefore the cognitive tools did not test every aspect of cognitive function and intelligence in minute detail, but focussed on collecting information in major domains in ~20 mins. A future larger study might rectify this shortcoming, especially as information processing speeds appear to be a specific neurological problem in SLE patients[317] and this should be investigated in greater detail, including associations with brain imaging data.

Final synthesis of main points and suggestions for future work

This thesis set out to investigate the role of inflammation in cerebral SVD. Inflammation has been seen pathologically in the perforating arteriolar walls and perivascular tissue in SVD since the 1800s, but the origin of the inflammation, systemic or intrinsic or both, is unclear. We have shown, for the first time, an increased burden of enlarged PVS as a marker of inflammation in a group of patients with a

systemic chronic inflammatory disease versus healthy controls and stroke patients with overt SVD, suggesting that inflammation is associated with changes consistent with SVD in the brain. We did not show conclusive evidence for an association between raised clinical inflammatory markers and SVD, although this does not preclude an association because inflammatory markers are best detected during active flares (which is difficult as large samples are required), whereas brain imaging evidence of the resulting damage is easily detected as it is thought to persist and accumulate. We also set out to clarify the association between inflammatory rheumatic diseases and stroke, including stroke subtypes and SVD, and although existing knowledge already suggests that rheumatic patients are at increased cardiovascular risk, we found that in general stroke occurred about two decades earlier in life than in patients without inflammatory rheumatic diseases, which is an important message to start stroke prevention strategies early.

It remains to be discovered in detail to what extent systemic inflammation contributes to SVD initiation and progression in the presence/absence of vascular risk factors and other environmental influences, and how inflammation interacts with traditional risk factors to accelerate SVD in some people but not others. Future studies should attempt to measure precisely and localise anatomically the exact location of the inflammation in the brain in SVD, possibly via use of advanced neuroinflammation imaging techniques (for a recent review of these techniques in dementia see Table 1 in Stefaniak and O'Brien[52]). Such techniques offer the potential to deepen our understanding of how inflammatory cells contribute to brain damage within the cerebral microcirculation in different diseases.

Interventions that dampen inflammation (pharmaceutically or via diet and lifestyle) in SVD are needed to see if this reduces disease burden and prevents recurrent vascular events in those that have already had a lacunar stroke or who are otherwise at risk of SVD. Brain imaging studies involving a range of different rheumatic patients are likely to be helpful in understanding how systemic inflammation affects the brain, e.g., in terms of localised or global dysfunction, and how the damage accumulates relative to systemic inflammation, including inflammatory flares and remissions. More work to raise public awareness of the potential damaging effects of inflammation on the brain would be beneficial.

Common sense suggests that patients with inflammatory rheumatic diseases should be encouraged to adopt healthy lifestyles and actively monitored for vascular risk factors and carefully managed within current guidelines. This may help prevent stroke but also mitigate long term accumulated brain damage thus helping to preserve cognition and wellbeing. The efficacy of such common sense approaches would require testing in randomized controlled trials.

References

1. Warlow C, Sudlow C, Dennis M, et al. Stroke. *Lancet* 2003;362:1211–24.

2. Feigin VL, Forouzanfar MH, Krishnamurthi R, et al. Global and regional burden of stroke during 1990-2010: Findings from the Global Burden of Disease Study 2010. *Lancet* 2014;383:245–55.

3. World Health Organization. Global health risks: mortality and burden of disease attributable to selected major risks. Bulletin of the World Health Organization 2009.

4. World Health Organization. The top 10 causes of death. Fact Sheet No. 310. 2014. Available from http://www.who.int/mediacentre/factsheets/fs310/en/ (Accessed 25 Feb 2016).

5. Krishnamurthi RV, Moran AE, Feigin VL, et al. Stroke prevalence, mortality and disability-adjusted life years in adults aged 20-64 years in 1990-2013: data from the Global Burden of Disease 2013 study. *Neuroepidemiol* 2015;45:190–202.

6. Mozaffarian D, Benjamin EJ, Go AS, et al. Heart disease and stroke statistics–2015 update: a report from the American Heart Association. *Circulation* 2015;131:e1–294.

7. Threapleton DE, Burley VJ, Greenwood DC, et al. Dietary fibre intake and risk of ischaemic and haemorrhagic stroke in the UK Women's Cohort Study. *Eur J Clin Nutrition* 2015;69:467–74.

8. Threapleton DE, Greenwood DC, Evans CEL, et al. Dietary fiber intake and risk of first stroke: A systematic review and meta-analysis. *Stroke* 2013;44:1360–8.

9. Chest Heart and Stroke Scotland. Stroke series SS1: stroke a guide to your recovery. 2007.

10. Wardlaw JM, Farrall AJ. Diagnosis of stroke on neuroimaging. *BMJ* 2004;328:655–6.

11. Grandin CB. Assessment of brain perfusion with MRI: methodology and application to acute stroke. *Neuroradiology* 2003;45:755–66.

12. Astrup J, Siesjo B, Symon L. Thresholds in cerebral ischemia – the ischemic penumbra. *Stroke* 1981;12:723–5.

13. Sarchielli P, Nardi K, Chiasserini D, et al. Immunological profile of silent brain infarction and lacunar stroke. *PLoS One* 2013;8:e68428.

14. Sudlow C, Warlow C. Comparable studies of the incidence of stroke and its pathological types: results from an international collaboration. International Stroke Incidence Collaboration. *Stroke* 1997;28:491–9.

15. Bamford JM, Sandercock P, Dennis M, et al. Classification and natural history of clinically identifiable subtypes of cerebral infarction. *Lancet* 1991;337:1521–26.

16. Pantoni L. Cerebral small vessel disease: from pathogenesis and clinical characteristics to therapeutic challenges. *Lancet Neurol* 2010;9:689–701.

17. Fisher CM. Lacunar strokes and infarcts: a review. *Neurology* 1982;32:871–6.

18. Wardlaw JM, Smith C, Dichgans M. Mechanisms of sporadic cerebral small vessel disease: insights from neuroimaging. *Lancet Neurol* 2013;12:483–97.

19. Wardlaw JM, Smith EE, Biessels GJ, et al. Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration. *Lancet Neurol* 2013;12:822–38.

20. Jackson C, Sudlow C. Are lacunar strokes really different? A systematic review of differences in risk factor profiles between lacunar and nonlacunar infarcts. *Stroke* 2005;36:891–901.

21. Debette S, Markus HS. The clinical importance of white matter hyperintensities on brain magnetic resonance imaging: systematic review and meta-analysis. *BMJ* 2010;341:c3666.

22. Gorelick PB, Scuteri A, Black SE, et al. Vascular contributions to cognitive impairment and dementia: A statement for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke* 2011;42:2672–713.

23. Pasi M, Salvadori E, Poggesi A, et al. White matter microstructural damage in small vessel disease is associated with Montreal Cognitive Assessment but not with Mini Mental State Examination performances: Vascular Mild Cognitive Impairment Tuscany Study. *Stroke* 2015;46:262–4.

24. Koga H, Takashima Y, Murakawa R, et al. Cognitive consequences of multiple lacunes and leukoaraiosis as vascular cognitive impairment in community-dwelling elderly individuals. *J Stroke Cerebrovasc Dis* 2009;18:32–7.

25. Herrmann LL, Le Masurier M, Ebmeier KP. White matter hyperintensities in late life depression: a systematic review. *J Neurol Neurosurg Psychiatry* 2008;79:619–24.

26. Valdés Hernández MDC, Booth T, Murray C, et al. Brain white matter damage in aging and cognitive ability in youth and older age. *Neurobiol Aging* 2013;34:2740–7.

27. Tuladhar AM, van Norden AGW, de Laat KF, et al. White matter integrity in small vessel disease is related to cognition. *NeuroImage: Clin* 2015;7:518–24.

28. Baezner H, Blahak C, Poggesi A, et al. Association of gait and balance disorders with age related white matter changes: the LADIS study. *Neurology* 2008;70:935–42.

29. Poggesi A, Pracucci G, Chabriat H, et al. on behalf of the LADIS Study Group. Urinary complaints in nondisabled elderly people with age-related white matter changes: the leukoaraiosis and disability (LADIS) study. *J Am Ger Soc* 2008;56:1638–43.

30. Suzuki Y, Nagai N, Umemura K. A review of the mechanisms of blood-brain barrier permeability by tissue-type plasminogen activator treatment for cerebral ischemia. *Frontiers Cell Neurosci* 2016;10:1–10.

31. Wardlaw JM, Sandercock PAG, Dennis MS, et al. Is breakdown of the blood-brain barrier responsible for lacunar stroke, leukoaraiosis, and dementia? *Stroke* 2003;34:806–12.

32. Wardlaw JM, Doubal F, Armitage P, et al. Lacunar stroke is associated with diffuse blood-brain barrier dysfunction. *Ann Neurol* 2009;65:194–202.

33. Davalos D, Ryu JK, Merlini M, et al. Fibrinogen-induced perivascular microglial clustering is required for the development of axonal damage in neuroinflammation. *Nature Comms* 2012;3:1227.

34. Charidimou A, Kakar P, Fox Z, et al. Cerebral microbleeds and recurrent stroke risk: systematic review and meta-analysis of prospective ischemic stroke and transient ischemic attack cohorts. *Stroke* 2013;44:995–1001.

35. Farrall AJ, Wardlaw JM. Blood-brain barrier: ageing and microvascular disease–systematic review and meta-analysis. *Neurobiol Aging* 2009;30:337–52.

36. Zhang J-M, An J. Cytokines, inflammation and pain. *Int Anesthesiol Clin* 2009;69:482–89.

37. Ghosh A, Birngruber T, Sattler W, et al. Assessment of blood-brain barrier function and the neuroinflammatory response in the rat brain by using cerebral Open Flow Microperfusion (cOFM). *PLoS One* 2014;9:3–9.

38. McColl BW, Rothwell NJ, Allan SM. Systemic inflammatory stimulus potentiates the acute phase and CXC chemokine responses to experimental stroke and exacerbates brain damage via interleukin-1- and neutrophil-dependent mechanisms. *J Neurosci* 2007;27:4403–12.

39. Zhang R, Chopp M, Jiang N. Anti–intercellular adhesion molecule–1 antibody reduces ischemic cell damage after transient but not permanent middle cerebral artery occlusion in the Wistar rat. *Stroke* 1995;26:1438–43.

40. The Enlimomab Acute Stroke Trial Investigators. Use of anti-ICAM-1 therapy in ischemic stroke: results of the Enlimomab Acute Stroke Trial. *Neurology* 2001;57:1428–34.

41. Ballabh P, Braun A, Nedergaard M. The blood-brain barrier: an overview: structure, regulation, and clinical implications. *Neurobiol Dis* 2004;16:1–13.

42. Perry VH. The impact of systemic inflammation on brain inflammation. *ACNR* 2004;4:8–9.

43. Libby P. Inflammation and atherosclerosis. *Circulation* 2002;105:1135–43.

44. Ross R. Atherosclerosis—An inflammatory disease. *New Eng J Med* 1999;340:115–26.

45. Van Leuven SI, Franssen R, Kastelein JJ, et al. Systemic inflammation as a risk factor for atherothrombosis. *Rheumatology* 2008;47:3–7.

46. Bailey EL, Smith C, Sudlow CLM, et al. Pathology of lacunar ischaemic stroke in humans–A systematic review. *Brain Pathol* 2012;22:583–91.

47. Lammie GA. Pathology of small vessel stroke. *Br Med Bull* 2000;56:296–306.

48. Aribisala BS, Wiseman S, Morris Z, et al. Circulating inflammatory markers are associated with magnetic resonance imaging-visible perivascular spaces but not directly with white matter hyperintensities. *Stroke* 2014;45:605–7.

49. Mitaki S, Nagai A, Oguro H et al. C-reactive protein levels are associated with cerebral small vessel-related lesions. *Acta Neurol Scan* 2015.

50. Hoogland ICM, Houbolt C, van Westerloo DJ, et al. Systemic inflammation and microglial activation: systematic review of animal experiments. *J Neuroinflammation* 2015;12:114.

51. Sutthiwarotamakun R. Peripheral autoimmunity induces central neuro-inflammation and hippocampal neurogenesis impairment in a murine model of collagen induced rheumatoid arthritis. 2011. Ph.D. thesis available from http://theses.gla.ac.uk/3027/ (Accessed 27 Nov 2014).

52. Stefaniak J, O'Brien J. Imaging of neuroinflammation in dementia: a review. *J Neurol Neurosurg Psychiatry* 2015;0:1–8.

53. Holmes C, Cunningham C, Zotova E, et al. Systemic inflammation and disease progression in Alzheimer disease. *Neurology* 2009;73:768–74.

54. National Institute of Arthritis and Musculoskeletal and Skin Diseases. Arthritis and rheumatic diseases. 2014. Available from http://www.niams.nih.gov/Health_Info/Arthritis/arthritis_rheumatic.pdf (Accessed 20 Mar 2016).

55. Holmqvist M, Simard JF, Asplund K, et al. Stroke in systemic lupus erythematosus: a meta-analysis of population-based cohort studies. *RMD Open* 2015;1:e000168.

56. Wiseman SJ, Ralston SH, Wardlaw JM. Cerebrovascular disease in rheumatic diseases: A systematic review and meta-analysis. *Stroke* 2016;47:943–50.

57. Bosma GPT, Huizinga TWJ, Mooijaart SP, et al. Abnormal brain diffusivity in patients with neuropsychiatric systemic lupus erythematosus. *AJNR Am J Neuroradiol* 2003;24:850–4.

58. Krupp LB, LaRocca NG, Muir-Nash J, et al. The fatigue severity scale. Application to patients with multiple sclerosis and systemic lupus erythematosus. *Arch Neurol* 1989;46:1121–3.

59. Carlomagno S, Migliaresi S, Ambrosone L, et al. Cognitive impairment in systemic lupus erythematosus: a follow-up study. *J Neurol* 2000;247:273–9.

60. Hewlett S, Nicklin J, Treharne G. Fatigue in musculoskeletal conditions. *J Rheumatol* Suppl. 2003;67:42–44.

61. Harboe E, Greve OJ, Beyer M, et al. Fatigue is associated with cerebral white matter hyperintensities in patients with systemic lupus erythematosus. *J Neurol Neurosurg Psychiatry* 2008;79:199–201.

62. Hamed SA, Selim ZI, Elattar AM, et al. Assessment of biocorrelates for brain involvement in female patients with rheumatoid arthritis. *Clin Rheumatol* 2012;31:123–32.

63. Atkinson AJJ, Colburn WA, DeGruttola VG, et al. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clin Pharma Therapeutics* 2001;69:89–95.

64. Roy S. Atherosclerotic cardiovascular disease risk and evidence-based management of cholesterol. *N Am J Med Sci* 2014;6:191–8.

65. Seshadri S, Beiser A, Selhub J, et al. Plasma homocysteine as a risk factor for dementia and Alzheimer's Disease. *New Eng J Med* 2002;346:476–83.

66. Beydoun MA, Beydoun HA, Gamaldo AA, et al. Epidemiologic studies of modifiable factors associated with cognition and dementia: systematic review and meta-analysis. *BMC Public Health* 2014;14:643.

67. Satizabal CL, Zhu YC, Mazoyer B, et al. Circulating IL-6 and CRP are associated with MRI findings in the elderly: the 3C-Dijon Study. *Neurology* 2012;78:720–7.

68. Knottnerus ILH, Ten Cate H, Lodder J, et al. Endothelial dysfunction in lacunar stroke: a systematic review. *Cerebrovasc Dis* 2009;27:519–26.

69. Markus HS, Hunt B, Palmer K, et al. Markers of endothelial and hemostatic activation and progression of cerebral white matter hyperintensities: longitudinal results of the Austrian Stroke Prevention Study. *Stroke* 2005;36:1410–14.

70. Stevenson SF, Doubal FN, Shuler K, et al. A systematic review of dynamic cerebral and peripheral endothelial function in lacunar stroke versus controls. *Stroke* 2010;41:e434–42.

71. Rouhl RPW, Damoiseaux JGMC, Lodder J, et al. Vascular inflammation in cerebral small vessel disease. *Neurobiol Aging* 2012;33:1800–6.

72. Ladenvall C, Jood K, Blomstrand C, et al. Serum C-reactive protein concentration and genotype in relation to ischemic stroke subtype. *Stroke* 2006;37:2018–23.

73. Montaner J, Perea-Gainza M, Delgado P, et al. Etiologic diagnosis of ischemic stroke subtypes with plasma biomarkers. *Stroke* 2008;39:2280–7.

74. Hasan N, McColgan P, Bentley P, et al. Towards the identification of blood biomarkers for acute stroke in humans: a comprehensive systematic review. *Br J Clin Pharmacol* 2012;74:230–40.

75. Moher D, Liberati A, Tetzlaff J, et al. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Medicine* 2009;6:e1000097.

76. Stroup DF, Berlin JA, Morton SC, et al. Meta-analysis of observational studies. *JAMA* 2000;283:2008–12.

77. Mischak H, Allmaier G, Apweiler R, et al. Recommendations for biomarker identification and qualification in clinical proteomics. *Sci Trans Med* 2010;2:46ps42.

78. McShane LM, Altman DG, Sauerbrei W, et al. REporting recommendations for tumour MARKer prognostic studies (REMARK). *Eur J Cancer* 2005;41:1690–6.

79. Von Elm E, Altman DG, Egger M, et al. [The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies]. *Revista española de salud pública* 2007;82:251–9.

80. Khan U, Crossley C, Kalra L, et al. Homocysteine and its relationship to stroke subtypes in a UK black population: the south London ethnicity and stroke study. *Stroke* 2008;39:2943–9.

81. Eikelboom JW, Hankey GJ, Anand SS, et al. Association between high homocyst(e)ine and ischaemic stroke due to large- and small-artery disease but not other etiologic subtypes of ischemic stroke. *Stroke* 2000;31:1069–75.

82. Hassan A, Hunt BJ, O'Sullivan M, et al. Homocysteine is a risk factor for cerebral small vessel disease, acting via endothelial dysfunction. *Brain* 2004;127:212–9.

83. Hanson E, Jood K, Karlsson S, et al. Plasma levels of von Willebrand factor in the etiologic subtypes of ischemic stroke. *J Thromb Haem* 2011;9:275–81.

84. Higgins J, Greens S. Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 [updated March 2011]. The Cochrane Collaboration, 2011. Available from www.cochrane-handbook.org (Accessed 14 Dec 2013).

85. Adams HP, Bendixen BH, Kappelle LJ, et al. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. *Stroke* 1993;24:35–41.

86. Kilpatrick TJ, Matkovic Z, Davis SM, et al. Hematologic abnormalities occur in both cortical and lacunar infarction. *Stroke* 1993;24:1945–50.

87. Castellanos M. Castillo J, Garcia MM, et al. Inflammation-mediated damage in progressing lacunar infarctions: a potential therapeutic target. *Stroke* 2002;33:982–87.

88. Domac FM, Somay G, Misirli H, et al. Tumor necrosis factor alpha serum levels and inflammatory response in acute ischemic stroke. *Neurosciences* 2006;12:25–30.

89. Beamer NB, Coull BM, Clark WM, et al. Interleukin-6 and interleukin-1 antagonist in acute stroke. *Ann Neurol* 1995;37:800–5.

90. Lindgren A, Lindoff C, Norrving B, et al. Tissue plasminogen activator and plasminogen activator inhibitor-1 in stroke patients. *Stroke* 1996;27:1066–71.

91. Salobir B, Sabovic M, Peternel P, et al. Classic risk factors, hypercoagulability and migraine in young women with cerebral lacunar infarctions. *Acta Neurol Scand* 2002;105:189–95.

92. Jood K, Ladenvall P, Tjärnlund-Wolf A, et al. Fibrinolytic gene polymorphism and ischemic stroke. *Stroke* 2005;36:2077–81.

93. Tuttolomondo A, Di Sciacca R, Di Raimondo D, et al. Plasma levels of inflammatory and thrombotic/fibrinolytic markers in acute ischemic strokes: relationship with TOAST subtype, outcome and infarct site. *J Neuroimmunology* 2009;215:84–9.

94. Tuttolomondo A, Pinto A, Corrao S, et al. Immuno-inflammatory and thrombotic/fibrinolytic variables associated with acute ischemic stroke diagnosis. *Atherosclerosis* 2009;203:503–8.

95. Yokokawa H, Goto A, Terui K, et al. Prevalence of metabolic syndrome and serum marker levels in patients with four subtypes of cerebral infarction in Japan. *J Clin Neuroscience* 2008;15:769–73.

96. Ilhan D, Ozbabalik D, Gulcan E, et al. Evaluation of platelet activation, coagulation, and fibrinolytic activation in patients with symptomatic lacunar stroke. *The Neurologist* 2010;16:188–91.

97. Bath PMW, Blann A, Smith N, et al. Von Willebrand Factor, P-selectin and fibrinogen levels in patients with acute ischaemic and haemorrhagic stroke, and their relationship with stroke sub-type and functional outcome. *Platelets* 1998;9:155–9.

98. Kataoka S, Hirose G, Hori A, et al. Activation of thrombosis and fibrinolysis following brain infarction. *J Neurol Sci* 2000;181:82–8.

99. Jood K, Danielson J, Ladenvall C, et al. Fibrinogen gene variation and ischemic stroke. *J Thromb Haem* 2008;6:897–904.

100. Alvarez-Perez FJ, Castelo-Branco M, Alvarez-Sabin J. Usefulness of measurement of fibrinogen, D-dimer, D-dimer/fibrinogen ratio, C reactive protein and erythrocyte sedimentation rate to assess the pathophysiology and mechanism of ischaemic stroke. *J Neurol Neurosurg Psychiatry* 2011;82:986–92.

101. Beer C, Blacker D, Hankey GJ, et al. Association of clinical and aetiologic subtype of acute ischaemic stroke and inflammation, oxidative stress and vascular function: a cross-sectional observational study. *Med Sci Monitor* 2011;17:467–73.

102. Zhang B, Zhang W, Li X, et al. Admission markers predict lacunar and non-lacunar stroke in young patients. *Thromb Research* 2011;128:14–7.

103. Takano K, Yamaguchi T, Uchida K. Markers of a hypercoagulable state following acute ischemic stroke. *Stroke* 1992;23:194–8.

104. Ageno W, Finazzi S, Steidl L, et al. Plasma measurement of D-dimer levels for the early diagnosis of ischemic stroke subtypes. *Arch Int Med* 2002;162:2589–93.

105. Brouns R, Van den Bossche J, De Surgeloose D, et al. Clinical and biochemical diagnosis of small-vessel disease in acute ischemic stroke. *J Neurol Sci* 2009;285:185–90.

106. Isenegger J, Meier N, Lämmle B, et al. D-dimers predict stroke subtype when assessed early. *Cerebrovasc Dis* 2010;29:82–6.

107. Parnetti L, Caso V, Santucci A, et al. Mild hyperhomocysteinemia is a risk-factor in all etiological subtypes of stroke. *Neurol Sci* 2004;25:13–7.

108. Khan U, Hassan A, Vallance P, et al. Asymmetric dimethylarginine in cerebral small vessel disease. *Stroke* 2007;38:411–3.

109. Jeong S-K, Kim D-H, Cho YI. Homocysteine and pulsatility index in lacunar infarction. *Clin Neurol Neurosurg* 2011;113:459–63.

110. Pavlovic AM, Pekmezovic T, Obrenovic R, et al. Increased total homocysteine level is associated with clinical status and severity of white matter changes in symptomatic patients with subcortical small vessel disease. *Clin Neurol Neurosurg* 2011;113:711–5.

111. Kozuka K, Kohriyama T, Nomura E, et al. Endothelial markers and adhesion molecules in acute ischemic stroke–sequential change and differences in stroke subtype. *Atherosclerosis* 2002;161:161–8.

112. Licata G, Tuttolomondo A, Di Raimondo D, et al. Immuno-inflammatory activation in acute cardio-embolic strokes in comparison with other subtypes of ischaemic stroke. *Thromb Haem* 2009;101:929–37.

113. Tsai N-W, Chang W-N, Shaw C-F, et al. Levels and value of platelet activation markers in different subtypes of acute non-cardio-embolic ischemic stroke. *Thrombosis Research* 2009;124:213–8.

114. Turgut B, Turgut N, Celik Y, et al. Differences in platelet-leukocyte aggregates among subtypes of acute cerebral ischemia. *J Neurol Sci* 2011;305:126–30.

115. Hassan A, Hunt BJ, O'Sullivan M, et al. Markers of endothelial dysfunction in lacunar infarction and ischaemic leukoaraiosis. *Brain* 2003;126:424–32.

116. Supanc V, Biloglav Z, Kes VB, et al. Role of cell adhesion molecules in acute ischemic stroke. *Ann Saudi Med* 2011;31:365–70.

117. Nakase T, Yamazaki T, Ogura N, et al. The impact of inflammation on the pathogenesis and prognosis of ischemic stroke. *J Neurol Sci* 2008;271:104–9.

118. Vila N, Castillo J, Davalos A, et al. Proinflammatory cytokines and early neurological worsening in ischemic stroke. *Stroke* 2000;31:2325–29.

119. Salobir B, Sabovic M. Possible vascular-bed-specific role of interleukin-6 in young women with a history of myocardial infarction, lacunar cerebral infarction and deep vein thrombosis. *Cytokine* 2004;25:265–72.

120. Guldiken B, Guldiken S, Turgut B, et al. The roles of oxidized low-density lipoprotein and interleukin-6 levels in acute atherothrombotic and lacunar ischemic stroke. *Angiology* 2008;59:224–9.

121. Potter GM, Marlborough FJ, Wardlaw JM. Wide variation in definition, detection, and description of lacunar lesions on imaging. *Stroke* 2011;42:359–66.

122. Fassbender K, Rossol S, Kammer T, et al. Proinflammatory cytokines in serum of patients with acute cerebral ischemia: kinetics of secretion and relation to the extent of brain damage and outcome of disease. *J Neurol Sci* 1994;122:135–9.

123. Ahmad O, Wardlaw J, Whiteley WN. Correlation of levels of neuronal and glial markers with radiological measures of infarct volume in ischaemic stroke: a systematic review. *Cerebrovasc Dis* 2012;33:47–54.

124. Whiteley W, Chong WL, Sengupta A, et al. Blood markers for the prognosis of ischemic stroke: a systematic review. *Stroke* 2009;40:e380–9.

125. Castellanos M, Serena J. Applicability of biomarkers in ischemic stroke. *Cerebrovasc Dis* 2007;24:7–15.

126. Wardlaw JM, Doubal FN, Valdes-Hernandez M, et al. Blood-brain barrier permeability and long-term clinical and imaging outcomes in cerebral small vessel disease. *Stroke* 2013;44:525–7.

127. Wiseman S, Marlborough F, Doubal F, et al. Blood markers of coagulation, fibrinolysis, endothelial dysfunction and inflammation in lacunar stroke versus non-lacunar stroke and non-stroke: systematic review and meta-analysis. *Cerebrovasc Dis* 2014;37:64–75.

128. Van Dijk EJ, Prins ND, Vermeer SE, et al. C-reactive protein and cerebral small-vessel disease: the Rotterdam Scan Study. *Circulation* 2005;112:900–5.

129. Wersching H, Duning T, Lohmann H, et al. Serum C-reactive protein is linked to cerebral microstructural integrity and cognitive function. *Neurology* 2010;74:1022–9.

130. Schmidt R, Schmidt H, Pichler M, et al. C-reactive protein, carotid atherosclerosis, and cerebral small-vessel disease: results of the Austrian Stroke Prevention Study. *Stroke* 2006;37:2910–16.

131. Shoamanesh A, Preis SR, Beiser AS, et al. Inflammatory biomarkers, cerebral microbleeds, and small vessel disease. Framingham Heart Study. *Neurology* 2015;84:825–32.

132. Nagai M, Hoshide S, Kario K. Association of prothrombotic status with markers of cerebral small vessel disease in elderly hypertensive patients. *Am J Hypertens* 2012;25:1088–94.

133. Kim J-S, Lee H-S, Park H-Y, et al. Endothelial function in lacunar infarction: a comparison of lacunar infarction, cerebral atherosclerosis and control group. *Cerebrovasc Dis* 2009;28:166–70.

134. Doubal FN, Dhillon B, Dennis MS, et al. Retinopathy in ischemic stroke subtypes. *Stroke* 2009;40:389–93.

135. Brott T, Adams HP, Olinger CP, et al. Measurements of acute cerebral infarction: a clinical examination scale. *Stroke* 1989;20:864–70.

136. Mead GE, Lewis SC, Wardlaw JM, et al. How well does the Oxfordshire Community Stroke Project classification predict the site and size of the infarct on brain imaging? *J Neurol Neurosurg Psychiatry* 2000;68:558–62.

137. Fazekas F, Chawluk JB, Alavi A, et al. MR Signal abnormalities at 1.5T in Alzheimer's dementia and normal aging. *Am J Roentgenol* 1987;149:351–6.

138. Potter G, Morris Z, Wardlaw J. Enlarged perivascular spaces (EPVS): a visual rating scale and user guide. Available at: http://www.bric.ed.ac.uk/documents/epvs-rating-scale-user-guide.pdf (Accessed 23 Mar 2014).

139. Cordonnier C, Potter GM, Jackson C, et al. Improving interrater agreement about brain microbleeds: development of the Brain Observer MicroBleed Scale (BOMBS). *Stroke* 2009;40:94–9.

140. Hernández MDCV, Ferguson KJ, Chappell FM, et al. New multispectral MRI data fusion technique for white matter lesion segmentation: method and comparison with thresholding in FLAIR images. *Eur Radiol* 2010;20:1684–91.

141. R Core Team: R: A language and environment for statistical computing. 2013. R Foundation for Statistical Computing, Vienna, Austria.

142. Gardner MJ, Altman DG. Statistics in medicine confidence intervals rather than P values: estimation rather than hypothesis testing. *BMJ* 1986;292:746–50.

143. Willeit P, Thompson A, Aspelund T, et al. Hemostatic factors and risk of coronary heart disease in general populations: new prospective study and updated meta-analyses. *PLoS One* 2013;8:e55175.

144. DeBono D. Significance of raised plasma concentrations of tissue-type plasminogen activator and plasminogen activator inhibitor in patients at risk from ischaemic heart disease. *Br Heart J* 1994;71:504–7.

145. Chandler WL, Jascur ML, Henderson PJ. Measurement of different forms of tissue plasminogen activator in plasma. *Clin Chem* 2000;46:38–46.

146. The IST-3 Collaborative Group. The benefits and harms of intravenous thrombolysis with recombinant tissue plasminogen activator within 6 h of acute ischaemic stroke (the third international stroke trial [IST-3]): a randomised controlled trial. *Lancet* 2012;379:2352–63.

147. Kvistad CE, Oeygarden H, Logallo N, et al. Is smoking associated with favourable outcome in tPA-treated stroke patients? *Acta Neurol Scand* 2014;130:299–304.

148. Kufner A, Nolte CH, Galinovic I, et al. Smoking-thrombolysis paradox: recanalization and reperfusion rates after intravenous tissue plasminogen activator in smokers with ischemic stroke. *Stroke* 2013;44:407–13.

149. Aries MJH, Uyttenboogaart M, Koch MW, et al. Does smoking influence outcome after intravenous thrombolysis for acute ischaemic stroke? *Eur J Neurol* 2009;16:819–22.

150. Knottnerus ILH, Govers-Riemslag JWP, Hamulyak K, et al. Endothelial activation in lacunar stroke subtypes. *Stroke* 2010;41:1617–22.

151. Baune BT, Ponath G, Rothermundt M, et al. Association between cytokines and cerebral MRI changes in the aging brain. *J Geriatr Psychiatry Neurol* 2009;22:23–34.

152. NHS National Services Scotland–Information Services Division. Stroke Statistics Update 2015:1–43.

153. Koton S, Schneider ALC, Rosamond WD, et al. Stroke incidence and mortality trends in US communities, 1987 to 2011. *JAMA* 2014;312:259–68.

154. JBS3 Board. Joint British Societies' consensus recommendations for the prevention of cardiovascular disease (JBS3). *Heart* 2014;100:ii1–ii67.

155. Peters MJL, Symmons DPM, McCarey D, et al. EULAR evidence-based recommendations for cardiovascular risk management in patients with rheumatoid arthritis and other forms of inflammatory arthritis. *Ann Rheum Dis* 2010;69:325–31.

156. Gabriel SE. Why do people with rheumatoid arthritis still die prematurely? *Ann Rheum Dis* 2008;67:iii30–iii34.

157. Yurkovich M, Vostretsova K, Chen W, et al. Overall and cause-specific mortality in patients with systemic lupus erythematosus: a meta-analysis of observational studies. *Arthritis Care Res* 2014;66:608–16.

158. Lindhardsen J, Ahlehoff O, Gislason GH, et al. Risk of atrial fibrillation and stroke in rheumatoid arthritis: Danish nationwide cohort study. *BMJ* 2012;344:e1257.

159. Solomon DH, Goodson NJ, Katz JN, et al. Patterns of cardiovascular risk in rheumatoid arthritis. *Ann Rheum Dis* 2006;65:1608–12.

160. Bessant R, Hingorani A, Patel L, et al. Risk of coronary heart disease and stroke in a large British cohort of patients with systemic lupus erythematosus. *Rheumatology* 2004;43:924–9.

161. Ward MM. Premature morbidity from cardiovascular and cerebrovascular diseases in women with systemic lupus erythematosus. *Arthritis Rheum* 1999;42:338–46.

162. Wang I-K, Muo C-H, Chang Y-C, et al. Risks, subtypes, and hospitalization costs of stroke among patients with systemic lupus erythematosus: a retrospective cohort study in Taiwan. *J Rheumatology* 2012;39:1611–18.

163. Holmqvist M, Gränsmark E, Mantel A, et al. Occurrence and relative risk of stroke in incident and prevalent contemporary rheumatoid arthritis. *Ann Rheum Dis* 2013;72:541–6.

164. Seminog OO, Goldacre MJ. Gout as a risk factor for myocardial infarction and stroke in England: evidence from record linkage studies. *Rheumatology* 2013;52:2251–59.

165. Szabo SM, Levy AR, Rao SR, et al. Increased risk of cardiovascular and cerebrovascular diseases in individuals with ankylosing spondylitis: a population-based study. *Arthritis Rheum* 2011;63:3294–304.

166. Mok CC, Ho LY, To CH. Annual incidence and standardized incidence ratio of cerebrovascular accidents in patients with systemic lupus erythematosus. *Scan J Rheum* 2009;38:362–8.

167. Turesson C. Cardiovascular co-morbidity in rheumatic diseases. *Vasc Health Risk Manag* 2008;4:605–14.

168. Esdaile JM, Abrahamowicz M, Grodzicky T, et al. Traditional Framingham risk factors fail to fully account for accelerated atherosclerosis in systemic lupus erythematosus. *Arthritis Rheum* 2001;44:2331–7.

169. Bamford J, Sandercock P, Jones L, et al. The natural history of lacunar infarction: the Oxfordshire Community Stroke Project. *Stroke* 1987;18:545–51.

170. Meune C, Touzé E, Trinquart L, et al. High risk of clinical cardiovascular events in rheumatoid arthritis: Levels of associations of myocardial infarction and stroke through a systematic review and meta-analysis. *Arch Cardiovasc Dis* 2010;103:253–61.

171. Rahman MM, Kopec JA, Cibere J, et al. The relationship between osteoarthritis and cardiovascular disease in a population health survey: a cross-sectional study. *BMJ Open* 2013;3:e002624.

172. Watson DJ, Rhodes T, Guess H. All-cause mortality and vascular events among patients with rheumatoid arthritis, osteoarthritis, or no arthritis in the UK General Practice Research Database. *J Rheumatol* 2003;30:1196–202.

173. Hak AE, Karlson EW, Feskanich D, et al. Systemic lupus erythematosus and risk of cardiovascular disease Results from the Nurses' Health Study. *Arthritis Rheum* 2010;61:1396–1402.

174. Krishnan E. Stroke subtypes among young patients with systemic lupus erythematosus. *Am J Med* 2005;118:1415e1-e7.

175. Liou T-H, Huang S-W, Lin J-W, et al. Risk of stroke in patients with rheumatism: a nationwide longitudinal population-based study. *Sci Rep* 2014;4:5110.

176. Mathieu S, Pereira B, Soubrier M. Cardiovascular events in ankylosing spondylitis: An updated meta-analysis. *Sem Arthritis Rheum* 2014:1–5.

177. Keller JJ, Hsu J-L, Lin S-M, et al. Increased risk of stroke among patients with ankylosing spondylitis: a population-based matched-cohort study. *Rheumatol Int* 2014;34:255–63.

178. Zöller B, Li X, Sundquist J, et al. Risk of subsequent ischemic and hemorrhagic stroke in patients hospitalized for immune-mediated diseases: a nationwide follow-up study from Sweden. *BMC Neurol* 2012;12:41.

179. Haroon NN, Paterson JM, Li P, et al. Patients with ankylosing spondylitis have increased cardiovascular and cerebrovascular mortality: A population-based study. *Ann Intern Med* 2015;163:409–16.

180. Ogdie A, Yu Y, Haynes K, et al. Risk of major cardiovascular events in patients with psoriatic arthritis, psoriasis and rheumatoid arthritis: a population-based cohort study. *Ann Rheum Dis* 2015;74:326–32.

181. Li W-Q, Han J-L, Manson JE, et al. Psoriasis and risk of nonfatal cardiovascular disease in U.S. women: a cohort study. *Br J Dermatol* 2012;166:811–8.

182. Kaye JA., Li L, Jick SS. Incidence of risk factors for myocardial infarction and other vascular diseases in patients with psoriasis. *Br J Dermatol* 2008;159:895–902.

183. Gelfand JM, Dommasch E, Shin DB, et al. Risk of stroke in patients with psoriasis. *J Invest Dermatol* 2010;129:2411–18.

184. Brauchli YB, Jick SS, Miret M, et al. Psoriasis and risk of incident myocardial infarction, stroke or transient ischaemic attack: an inception cohort study with a nested case-control analysis. *Br J Dermatol* 2009;160:1048–56.

185. Yang Y-W, Keller JJ, Lin H-C. Medical comorbidity associated with psoriasis in adults: a population-based study. *Br J Dermatol* 2011;165:1037–43.

186. Kimball AB, Robinson D, Wu Y, et al. Cardiovascular disease and risk factors among psoriasis patients in two US healthcare databases, 2001-2002. *Dermatol* 2008;217:27–37.

187. Han C, Robinson DW, Hackett MV, et al. Cardiovascular disease and risk factors in patients with rheumatoid arthritis, psoriatic arthritis, and ankylosing spondylitis. *J Rheumatol* 2006;33:2167–72.

188. Gladman DD, Ang M, Su L, et al. Cardiovascular morbidity in psoriatic arthritis. *Ann Rheum Dis* 2009;68:1131–5.

189. Ahlehoff O, Gislason GH, Charlot M, et al. Psoriasis is associated with clinically significant cardiovascular risk: a Danish nationwide cohort study. *J Int Med* 2011;270:147–57.

190. Dowlatshahi EA, Kavousi M, Nijsten T, et al. Psoriasis is not associated with atherosclerosis and incident cardiovascular events: the Rotterdam Study. *J Invest Dermatol* 2013;133:2347–54.

191. Schmitt J, Ford DE. Psoriasis is independently associated with psychiatric morbidity and adverse cardiovascular risk factors, but not with cardiovascular events in a population-based sample. *J Eur Acad Dermatol Venereol* 2010;24:885–92.

192. Chin Y-Y, Yu H-S, Li W-C, et al. Arthritis as an important determinant for psoriatic patients to develop severe vascular events in Taiwan: a nation-wide study. *J Eur Acad Dermatol Venereol* 2013;27:1262–8.

193. Horreau C, Pouplard C, Brenaut E, et al. Cardiovascular morbidity and mortality in psoriasis and psoriatic arthritis: a systematic literature review. *J Eur Acad Dermatol Venereol* 2013;27:12–29.

194. Armstrong EJ, Harskamp CT, Armstrong AW. Psoriasis and major adverse cardiovascular events: a systematic review and meta-analysis of observational studies. *J Am Heart Assoc* 2013;2:e000062.

195. Low AS, Lunt M, Mercer LK, et al. Association between anti-tumor necrosis factor therapy and the risk of ischemic stroke in subjects with rheumatoid arthritis. Results from the British Society for Rheumatology Biologics Registers-Rheumatoid Arthritis (BSRBR-RA). [abstract]. *Arthritis Rheum* 2012;64.

196. Turesson C, Jarenros A, Jacobsson L. Increased incidence of cardiovascular disease in patients with rheumatoid arthritis: results from a community based study. *Ann Rheum Dis* 2004;63:952–5.

197. Semb AG, Kvien TK, Aastveit AH, et al. Lipids, myocardial infarction and ischaemic stroke in patients with rheumatoid arthritis in the Apolipoprotein-related Mortality RISk (AMORIS) Study. *Ann Rheum Dis* 2010;69:1996–2001.

198. Nadareishvili Z, Michaud K, Hallenbeck JM, et al. Cardiovascular, rheumatologic, and pharmacologic predictors of stroke in patients with rheumatoid arthritis: a nested, case-control study. *Arthritis Rheum* 2008;59:1090–6.

199. Mikdashi J, Handwerger B, Langenberg P, et al. Baseline disease activity, hyperlipidemia, and hypertension are predictive factors for ischemic stroke and stroke severity in systemic lupus erythematosus. *Stroke* 2007;38:281–5.

200. Gustafsson J, Gunnarsson I, Börjesson O, et al. Predictors of the first cardiovascular event in patients with systemic lupus erythematosus – a prospective cohort study. *Arthritis Res Ther* 2009;11:R186.

201. Chiu C-C, Huang C-C, Chan W-L, et al. Increased risk of ischemic stroke in patients with systemic lupus erythematosus: A nationwide population-based study. *Int Med* 2012;51:17–21.

202. Rothwell PM, Coull AJ, Silver LE, et al. Population-based study of event-rate, incidence, case fatality, and mortality for all acute vascular events in all arterial territories (Oxford Vascular Study). *Lancet* 2005;366:1773–83.

203. Sacco S, Marini C, Totaro R, et al. A population-based study of the incidence and prognosis of lacunar stroke. *Neurology* 2006;66:1335–38.

204. Kaichi Y, Kakeda S, Moriya J, et al. Brain MR findings in patients with systemic lupus erythematosus with and without antiphospholipid antibody syndrome. *AJNR Am J Neuroradiol* 2014;35:100–5.

205. Appenzeller S, Rondina JM, Li LM, et al. Cerebral and corpus callosum atrophy in systemic lupus erythematosus. *Arthritis Rheum* 2005;52:2783–9.

206. Appenzeller S, Bonilha L, Rio PA, et al. Longitudinal analysis of gray and white matter loss in patients with systemic lupus erythematosus. *NeuroImage* 2007;34:694–701.

207. Appenzeller S, Vasconcelos Faria A, Li LM, et al. Quantitative magnetic resonance imaging analyses and clinical significance of hyperintense white matter lesions in systemic lupus erythematosus patients. *Ann Neurol* 2008;64:635–43.

208. Valdés-Ferrer SI, Vega F, Cantú-Brito C, et al. Cerebral changes in SLE with or without antiphospholipid syndrome. A case-control MRI study. *J Neuroimaging* 2008;18:62–5.

209. Appenzeller S, Carnevalle AD, Li LM, et al. Hippocampal atrophy in systemic lupus erythematosus. *Ann Rheum Dis* 2006;65:1585–9.

210. Akasbi M, Berenguer J, Saiz A, et al. White matter abnormalities in primary Sjögren syndrome. *Q J Med* 2012;105:433–43.

211. Steup-Beekman GM, Zirkzee EJM, Cohen D, et al. Neuropsychiatric manifestations in patients with systemic lupus erythematosus: epidemiology and radiology pointing to an immune-mediated cause. *Ann Rheum Dis* 2013;72:ii76–9.

212. Katsumata Y, Harigai M, Kawaguchi Y, et al. Diagnostic reliability of magnetic resonance imaging for central nervous system syndromes in systemic lupus erythematosus: a prospective cohort study. *BMC Musculoskelet Disord* 2010;11:13.

213. Jennings JE, Sundgren PC, Attwood J, et al. Value of MRI of the brain in patients with systemic lupus erythematosus and neurologic disturbance. *Neuroradiology* 2004;46:15–21.

214. Sanna G, Piga M, Terryberry JW, et al. Central nervous system involvement in systemic lupus erythematosus: cerebral imaging and serological profile in patients with and without overt neuropsychiatric manifestations. *Lupus* 2000;9:573–83.

215. Sudlow C, Warlow CP. Comparing stroke incidence worldwide. *Stroke* 1996;27:550–8.

216. Bos MJ, Koudstaal PJ, Hofman A, et al. Uric acid is a risk factor for myocardial infarction and stroke: the Rotterdam study. *Stroke* 2006;37:1503–7.

217. Lindsberg PJ, Grau AJ. Inflammation and infections as risk factors for ischemic stroke. *Stroke* 2003;34:2518–32.

218. Saidi S, Mahjoub T, Almawi WY. Lupus anticoagulants and anti-phospholipid antibodies as risk factors for a first episode of ischemic stroke. *J Thromb Haemost* 2009;7:1075–80.

219. Sciascia S, Sanna G, Khamashta MA, et al. The estimated frequency of antiphospholipid antibodies in young adults with cerebrovascular events: a systematic review. *Ann Rheum Dis* 2015;74:2028–33.

220. Petri M, Roubenoff R, Dallal GE, et al. Plasma homocysteine as a risk factor for atherothrombotic events in systemic lupus erythematosus. *Lancet* 1996;348:1120–4.

221. Lee S, Shafe ACE, Cowie MR. UK stroke incidence, mortality and cardiovascular risk management 1999-2008: time-trend analysis from the General Practice Research Database. *BMJ Open* 2011;1:e000269.

222. Davenport R, Dennis M, Warlow C. The accuracy of Scottish Morbidity Record (SMR1) data for identifying hospitalised stroke patients. *Health Bull (Edinb)* 1996;54:402–5.

223. Lévy L, Fautrel B, Barnetche T, et al. Incidence and risk of fatal myocardial infarction and stroke events in rheumatoid arthritis patients. A systematic review of the literature. *Clin Exp Rheumatol* 2008;26:673–9.

224. Joseph FG, Scolding NJ. Neurolupus. *Pract Neurol* 2010;10:4–15.

225. Hanly JG, Urowitz MB, Su L, et al. Prospective analysis of neuropsychiatric events in an international disease inception cohort of SLE patients. *Ann Rheum Dis* 2011;69:529–35.

226. Bosma GPT, Middelkoop HAM, Rood MJ, et al. Association of global brain damage and clinical functioning in neuropsychiatric systemic lupus erythematosus. *Arthritis Rheum* 2002;46:2665–72.

227. Penke L, Maniega SM, Bastin ME, et al. Brain white matter tract integrity as a neural foundation for general intelligence. *Mol Psychiatry* 2012;17:1026–30.

228. Zimny A, Szewczyk P, Bladowska J, et al. In vivo evaluation of brain damage in the course of systemic lupus erythematosus using magnetic resonance spectroscopy, perfusion-weighted and diffusion-tensor imaging. *Lupus* 2014;23:10–19.

229. Schmidt-Wilcke T, Cagnoli P, Wang P, et al. Diminished white matter integrity in patients with systemic lupus erythematosus. *NeuroImage Clin* 2014;5:291–7.

230. Zivadinov R, Shucard JL, Hussein S, et al. Multimodal imaging in systemic lupus erythematosus patients with diffuse neuropsychiatric involvement. *Lupus* 2013;22:675–83.

231. Jung RE, Caprihan A, Chavez RS, et al. Diffusion tensor imaging in neuropsychiatric systemic lupus erythematosus. *BMC Neurol* 2010;10:65.

232. Emmer BJ, Veer IM, Steup-Beekman GM, et al. Tract-based spatial statistics on diffusion tensor imaging in systemic lupus erythematosus reveals localized involvement of white matter tracts. *Arthritis Rheum* 2010;62:3716–21.

233. Welsh RC, Rahbar H, Foerster B, et al. Brain diffusivity in patients with neuropsychiatric systemic lupus erythematosus with new acute neurological symptoms. *J Magn Reson Imaging* 2007;26:541–51.

234. Hughes M, Sundgren PC, Fan X, et al. Diffusion tensor imaging in patients with acute onset of neuropsychiatric systemic lupus erythematosus: a prospective study of apparent diffusion coefficient, fractional anisotropy values, and eigenvalues in different regions of the brain. *Acta Radiol* 2007;48:213–22.

235. Zhang L, Harrison M, Heier LA, et al. Diffusion changes in patients with systemic lupus erythematosus. *Magn Reson Imaging* 2007;25:399–405.

236. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1997;40:1725.

237. Tan E, Cohen A, Fries J, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1982;25:1271–7.

238. Williams B, Poulter NR, Brown MJ, et al. British Hypertension Society guidelines for hypertension management 2004 (BHS-IV): summary. *BMJ* 2004;328:634–40.

239. Gladman D, Ibanez D, Urowitz M. Systemc lupus erythematosus disease activity index 2000. *J Rheumatol* 2002;29:288–91.

240. Isenberg DA, Rahman A, Allen E, et al. BILAG 2004. Development and initial validation of an updated version of the British Isles Lupus Assessment Group's disease activity index for patients with systemic lupus erythematosus. *Rheumatology* 2005;44:902–6.

241. Gladman D, Ginzler E, Goldsmith C, et al. The development and initial validation of the systemic lupus international collaborating clinics / America College of Rheumatology Damage Index for systemic lupus erythematosus. *Arthritis Rheum* 1996;39:363–9.

242. Gladman D, Goldsmith C, Urowitz M, et al. The Systemic Lupus International Collaborating Clinics/American College of Rheumatology (SLICC/ACR) Damage Index for Systemic Lupus Erythematosus International Comparison. *J Rheumatol* 2000;27:373–6.

243. Petri M. Disease activity assessment in SLE: do we have the right instruments? *Ann Rheum Dis* 2007;66 Suppl 3:iii61–4.

244. Bombardier C, Gladman DD, Urowitz MB, et al. and the Committee on Prognosis Studies in SLE. Derivation of the SLEDAI. A disease activity index for lupus patients. *Arthritis Rheum* 1992;35:630-40.

245. Symmons DPM, Coppock JS, Bacon PA, et al. Development and assessment of a computerized index of clinical disease activity in systemic lupus erythematosus. *Q J Med* 1988;69:927–37.

246. Yee C-S, Cresswell L, Farewell V, et al. Numerical scoring for the BILAG-2004 index. *Rheumatology* 2010;49:1665–9.

247. Dayal NA, Gordon C, Tucker L, et al. The SLICC damage index: past, present and future. *Lupus* 2002;11:261–5.

248. Zigmond A, Snaith R. The hospital anxiety and depression scale. *Acta Psychiatr Scand* 1983;67:361–70.

249. Bjelland I, Dahl AA, Haug TT, et al. The validity of the Hospital Anxiety and Depression Scale. An updated literaure review. *J Psychosomatic Res* 2002;52:69–77.

250. Narseddine Z, Phillips N, Bedirian V, et al. The Montreal Cognitive Assessment, MoCA : A brief screening tool for mild cognitive impairment. *J Am Geriatr Soc* 2005;53:695–9.

251. Hsieh S, Schubert S, Hoon C, et al. Validation of the Addenbrooke's Cognitive Examination III in frontotemporal dementia and Alzheimer's disease. *Dement Geriatr Cogn Disord* 2013;36:242–50.

252. Folstein M, Folstein S, McHugh P. Mini-mental state. A practical method for grading the cognitive state of patients for the clinician. *J Psychiat Res* 1975;12:189–98.

253. Nelson H, Willison J. National Adult Reading Test (NART):Test Manual. NFER_Nelson Publ 1982.

254. McGurn B, Starr JM, Topfer JA, et al. Pronunciation of irregular words is preserved in dementia, validating premorbid IQ estimation. *Neurology* 2004;62:1184–7.

255. Potter GM, Chappell FM, Morris Z, et al. Cerebral perivascular spaces visible on magnetic resonance imaging: development of a qualitative rating scale and its observer reliability. *Cerebrovasc Dis* 2015;39:224–31.

256. Valdés Hernández M del C, Armitage PA, Thrippleton MJ, et al. Rationale, design and methodology of the image analysis protocol for studies of patients with cerebral small vessel disease and mild stroke. *Brain Behav* 2015;0:e00415.

257. Farrell C, Chappell F, Armitage PA, et al. Development and initial testing of normal reference MR images for the brain at ages 65-70 and 75-80 years. *Eur Radiol* 2009;19:177–83.

258. Staals J, Makin SDJ, Doubal FN, et al. Stroke subtype, vascular risk factors, and total MRI brain small-vessel disease burden. *Neurology* 2014;83:1–7.

259. Klarenbeek P, van Oostenbrugge RJ, Rouhl RPW, et al. Ambulatory blood pressure in patients with lacunar stroke: association with total MRI burden of cerebral small vessel disease. *Stroke* 2013;44:2995–9.

260. Jenkinson M, Smith S. A global optimization method for robust affine registration of brain images. *Med Image Anal* 2001;5:143–56.

261. Wardlaw JM, Bastin ME, Valdés Hernández MC, et al. Brain aging, cognition in youth and old age and vascular disease in the Lothian Birth Cohort 1936: rationale, design and methodology of the imaging protocol. *Int J Stroke* 2011;6:547–59.

262. Perneger TV. What's wrong with Bonferroni adjustments. *BMJ* 1998;316:1236.

263. Jensen A. The g Factor: The science of mental ability. London: Praeger; 1998.

264. Piga M, Peltz MT, Montaldo C, et al. Twenty-year brain magnetic resonance imaging follow-up study in systemic lupus erythematosus: factors associated with accrual of damage and central nervous system involvement. *Autoimmun Rev* 2015;14:510–16.

265. Wuerfel J, Haertle M, Waiczies H, et al. Perivascular spaces – MRI marker of inflammatory activity in the brain? *Brain* 2008;131:2332–40.

266. Chung CP, Ormseth MJ, Connelly MA, et al. GlycA, a novel marker of inflammation, is elevated in systemic lupus erythematosus. *Lupus* 2016;25:296–300.

267. Ellis S, Verity M. Central nervous system involvement in systemic lupus erythematosus: a review of neuropathologic findings in 57 cases, 1955-1977. *Sem Arthritis Rheum* 1979;8:212–21.

268. Stimmler MM, Coletti PM, Quismorio FP. Magnetic resonance imaging of the brain in neuropsychiatric systemic lupus erythematosus. *Sem Arthritis Rheum* 1993;22:335–49.

269. Mikdashi JA, Mehta RI, Castellani RJ. Cerebral small vessel disease in systemic lupus erythematosus: histopathological study. [Abstract] ACR/ARHP Annual Meeting. 2014.

270. Keene D, Price C, Shun-Shin MJ, et al. Effect on cardiovascular risk of high density lipoprotein targeted drug treatments niacin, fibrates, and CETP inhibitors: meta-analysis of randomised controlled trials including 117,411 patients. *BMJ* 2014;349:g4379.

271. Zanoni P, Khetarpal SA, Larach DB, et al. Rare variant in scavanger receptor BI raises HDL cholesterol and increases risk of coronary heart disease. *Science* 2016;351:1166–71.

272. McMahon M, Skaggs BJ, Grossman JM, et al. A panel of biomarkers is associated with increased risk of the presence and progression of atherosclerosis in women with systemic lupus erythematosus. *Arthritis Rheum* 2014;66:130–9.

273. Wang X, Dong Y, Qi X, et al. Cholesterol levels and risk of hemorrhagic stroke: a systematic review and meta-analysis. *Stroke* 2013;44:1833–9.

274. Navab M, Berliner JA, Watson AD, et al. The yin and yang of oxidation in the development of the fatty streak. A review based on the 1994 George Lyman Duff memorial lecture. *Arterioscler Thromb Vasc Biol* 1996;16:831–42.

275. Navab M, Hama SY, Hough GP, et al. A cell-free assay for detecting HDL that is dysfunctional in preventing the formation of or inactivating oxidized phospholipids. *J Lipid Res* 2001;42:1308–17.

276. Vazquez E, Sethi AA, Freeman L, et al. High-density lipoprotein cholesterol efflux, nitration of apolipoprotein A-I and endothelial function in obese women. *Am J Cardiol* 2012;109:527–32.

277. Wu Q, Inman RD, Davis KD. Fatigue in ankylosing spondylitis is associated with the brain networks of sensory salience and attention. *Arthritis Rheum* 2014;66:295–303.

278. Basu N, Murray AD, Jones GT, et al. Fatigue-related brain white matter changes in granulomatosis with polyangiitis. *Rheumatology* 2013;52:1429–34.

279. Sinha S, Bastin ME, Wardlaw JM, et al. Effects of dexamethasone on peritumoural oedematous brain: a DT-MRI study. *J Neurol Neurosurg Psychiatry* 2004;75:1632–5.

280. Chaiamnuay S, Bertoli A, Fernandez M, et al. The impact of increased body mass index on systemic lupus erythematosus: Data from LUMINA, a multiethnic cohort. *J Clin Rheumatol* 2007;13:128–33.

281. Kellner ES, Lee PY, Li Y, et al. Endogenous type-I interferon activity is not associated with depression or fatigue in systemic lupus erythematosus. *J Neuroimmunol* 2010;223:13–19.

282. Omdal R, Mellgren S, Koldingsness W, et al. Fatigue in patients with systemic lupus erythematosus: lack of associations to serum cytokines, antiphospholipid antibodies, or other disease characteristics. *J Rheumatol* 2002;29:482–6.

283. Mooijaart SP, Sattar N, Trompet S, et al. Circulating interleukin-6 concentration and cognitive decline in old age: the PROSPER study. *J Intern Med* 2013;274:77–85.

284. Tzarouchi LC, Zikou AK, Tsifetaki N, et al. White matter water diffusion changes in Primary Sjogren Syndrome. *AJNR Am J Neuroradiol* 2014;35:680–5.

285. Wiseman S, Doubal FN, Chappell FM, et al. Plasma biomarkers of inflammation, endothelial function and haemostasis in cerebral small vessel disease. *Cerebrovasc Dis* 2015;40:157–164.

286. National Collaborating Centre for Chronic Conditions. Stroke: national clinical guideline for diagnosis and initial treatment of acute stroke and transient ischaemic (TIA). 2008. London: Royal College of Physicians.

287. He FJ, Nowson CA, MacGregor GA. Fruit and vegetable consumption and stroke: meta-analysis of cohort studies. *Lancet* 2006;367:320–6.

288. D'Elia L, Barba G, Cappuccio FP, et al. Potassium intake, stroke, and cardiovascular disease: A meta-analysis of prospective studies. *J Am Coll Cardiol* 2011;57:1210–19.

289. Hansson GK, Hermansson A. The immune system in atherosclerosis. *Nature Immunol* 2011;12:204–12.

290. McColl BW, Allan SM, Rothwell NJ. Systemic infection, inflammation and acute ischemic stroke. *Neuroscience* 2009;158:1049–61.

291. Moskowitz MA, Lo EH, Iadecola C. The science of stroke: mechanisms in search of treatments. *Neuron* 2010;67:181–98.

292. Vogel R, Corretti M, Plotnick G. Effect of a single high-fat meal on endothelial function in healthy subjects. *Am J Cardiol* 1997;79:350–4.

293. Bui C, Petrofsky J, Berk L, et al. Acute effect of a single high-fat meal on forearm blood flow, blood pressure and heart rate in healthy male Asians and Caucasians: A pilot study. *SE Asian J Tropical Med Pub Health* 2010;41:490–500.

294. Ghanim H, Abuaysheh S, Sia C, et al. Increase in plasma endotoxin concentrations and the expression of toll-like receptors and the suppressor of cytokine signaling-3 in mononuclear cells after a high-fat, high-carbohydrate meal. *Diabetes Care* 2009;32:2281–7.

295. Maysami S, Haley MJ, Gorenkova N, et al. Prolonged diet-induced obesity in mice modifies the inflammatory response and leads to worse outcome after stroke. *J Neuroinflamm* 2015;12:140.

296. Terao S, Yilmaz G, Stokes KY, et al. Inflammatory and injury responses to ischemic stroke in obese mice. *Stroke* 2008;39:943–50.

297. Benavente OR, Hart RG, McClure LA, et al. on behalf of the SPS3 Investigators. Effects of clopidogrel added to aspirin in patients with recent lacunar stroke. *New Eng J Med* 2012;367:817–25.

298. Elkind MSV, Luna JM, McClure LA, et al. C-reactive protein as a prognostic marker after lacunar stroke levels of inflammatory markers in the treatment of stroke study. *Stroke* 2014;45:707–16.

299. Boehme AK, McClure LA, Zhang Y, et al. Inflammatory markers and outcomes after lacunar stroke. *Stroke* 2016;47:659–67.

300. Fornage M, Chiang YA, O'Meara ES, et al. Biomarkers of inflammation and MRI-defined small vessel disease of the brain: the Cardiovascular Health Study. *Stroke* 2008;39:1952–9.

301. van Bussel BC, Schouten F, Henry RM, et al. Endothelial dysfunction and low-grade inflammation are associated with greater arterial stiffness over a 6-year period. *Hypertension* 2011;58:588–95.

302. Dod H, Bhardwaj R, Sajja V, et al. Effect of intensive lifestyle changes on endothelial function and on inflammatory markers of atherosclerosis. *Am J Cardiol* 2010;105:362–7.

303. Smith AD, Smith SM, de Jager CA, et al. Homocysteine-lowering by b vitamins slows the rate of accelerated brain atrophy in mild cognitive impairment: A randomized controlled trial. *PLoS One* 2010;5:1–10.

304. Jeon S-B, Kang D-W, Kim JS, et al. Homocysteine, small-vessel disease, and atherosclerosis: an MRI study of 825 stroke patients. *Neurology* 2014;83:695–701.

305. Bacon P, Townend J. Nails in the coffin: increasing evidence for the role of rheumatic disease in the cardiovascular mortality of rheumatoid arthritis. *Arthritis Rheum* 2001;44:2707–10.

306. Abou-Raya A, Abou-Raya S. Inflammation: a pivotal link between autoimmune diseases and atherosclerosis. *Autoimmunity Reviews* 2006;5:331–7.

307. Stern DM, Esposito C, Gerlach H, et al. Endothelium and regulation of coagulation. *Diabetes Care* 1991;14:160–6.

308. Van Hinsbergh VWM. Endothelium—role in regulation of coagulation and inflammation. *Sem Immunopathol* 2012;34:93–106.

309. Dixon WG, Watson KD, Lunt M, et al. Reduction in the incidence of myocardial infarction in patients with rheumatoid arthritis who respond to anti-tumor necrosis factor α therapy: Results from the British Society for Rheumatology Biologics Register. *Arthritis Rheum* 2007;56:2905–12.

310. Nidorf S, Eikelboom J, Budgeon C, et al. Low-dose colchicine for secondary prevention of cardiovascular disease. *J Am Coll Cardiol* 2013;61:404–10.

311. Tabas I, Glass CK. Anti-inflammatory therapy in chronic disease: challenges and opportunities. *Science* 2013;339:166–72.

312. Xiao Z, Pei Z, Yuan M, et al. Risk of stroke in patients with inflammatory bowel disease: a systematic review and meta-analysis. *J Stroke Cerebrovasc Dis* 2015;24:2774–80.

313. Yuan M, Zhou H-Y, Xiao X-L, et al. Inflammatory bowel disease and risk of stroke : a meta-analysis of cohort studies. *Intl J Cardiol* 2016;202:106–09.

314. Ferro JM, Oliveira SN, Correia L. Neurologic manifestations of inflammatory bowel diseases. *Handbook Clin Neurol* 2014;120:595–605.

315. Dolapcioglu C. Structural brain lesions in inflammatory bowel disease. *World J Gastroint Pathophysiol* 2015;6:124–130.

316. Zikou AK, Kosmidou M, Astrakas LG, et al. Brain involvement in patients with inflammatory bowel disease: a voxel-based morphometry and diffusion tensor imaging study. *Eur Radiol* 2014;24:2499–506.

317. Hanly J, Omisade A, Su L, et al. Cognitive function in systemic lupus erythematosus, rheumatoid arthritis and multiple sclerosis assessed by computerized neuropsychological tests. *Arhtirits Rheum* 2010;62:1478–86.

Appendix A: Copies of published papers

"Blood markers of coagulation, fibrinolysis, endothelial dysfunction and inflammation in lacunar stroke versus non-lacunar stroke and non-stroke: systematic review and meta-analysis" (see Chapter 2). Reproduced with permission from the publisher S. Karger AG.

Review

Cerebrovascular Diseases

Cerebrovasc Dis 2014;37:64–75
DOI: 10.1159/000356789

Received: August 8, 2013
Accepted: October 24, 2013
Published online: December 21, 2013

Blood Markers of Coagulation, Fibrinolysis, Endothelial Dysfunction and Inflammation in Lacunar Stroke versus Non-Lacunar Stroke and Non-Stroke: Systematic Review and Meta-Analysis

Stewart Wiseman Fergal Marlborough Fergus Doubal David J. Webb
Joanna Wardlaw

Brain Research Imaging Centre, University of Edinburgh, Edinburgh, UK

Key Words
Biomarker · Endothelium · Inflammation · Stroke · Lacunar stroke

Abstract
Background: The cause of cerebral small vessel disease is not fully understood, yet it is important, accounting for about 25% of all strokes. It also increases the risk of having another stroke and contributes to about 40% of dementias. Various processes have been implicated, including microatheroma, endothelial dysfunction and inflammation. A previous review investigated endothelial dysfunction in lacunar stroke versus mostly non-stroke controls while another looked at markers of inflammation and endothelial damage in ischaemic stroke in general. We have focused on blood markers between clinically evident lacunar stroke and other subtypes of ischaemic stroke, thereby controlling for stroke in general. ***Summary:*** We systematically assessed the literature for studies comparing blood markers of coagulation, fibrinolysis, endothelial dysfunction and inflammation in lacunar stroke versus non-stroke controls or other ischaemic stroke subtypes. We assessed the quality of included papers and meta-analysed results. We split the analysis on time of blood draw in relation to the stroke. We identified 1,468 full papers of which 42 were eligible for inclusion, including 4,816 ischaemic strokes, of which 2,196 were lacunar and 2,500 non-stroke controls. Most studies subtyped stroke using TOAST. The definition of lacunar stroke varied between studies. Markers of coagulation/fibrinolysis (tissue plasminogen activator (tPA), plasminogen activator inhibitor (PAI), fibrinogen, D-dimer) were higher in lacunar stroke versus non-stroke although fibrinogen was no different to non-stroke in the acute phase. tPA and PAI were no different between lacunar and non-lacunar stroke. Fibrinogen and D-dimer were significantly lower in lacunar stroke compared to other ischaemic strokes, both acutely and chronically. Markers of endothelial dysfunction (homocysteine, von Willebrand Factor (vWF), E-selectin, P-selectin, intercellular adhesion molecule-1 (ICAM), vascular cellular adhesion molecule-1 (VCAM)) were higher or had insufficient or conflicting data (P-selectin, VCAM) in lacunar stroke versus non-stroke. Compared to other ischaemic stroke subtypes, homocysteine did not differ in lacunar stroke while vWF was significantly lower in lacunar stroke acutely [atherothrombotic standardized mean difference, SMD, −0.34 (−0.61, −0.08); cardioembolic SMD −0.38 (−0.62, −0.14)], with insufficient data chronically. Markers of inflammation (C-reactive protein (CRP), tumour necrosis factor-alpha (TNF-α), interleukin-6 (IL-6)) were higher in lacunar stroke versus non-stroke, although there were no studies measuring TNF-α chronically and the sole study measuring IL-6 chroni-

Joanna Wardlaw, FRCP, FRCR, FMedSci
SINAPSE Collaboration, Brain Research Imaging Centre
Neuroimaging Sciences, Western General Hospital
Crewe Road, Edinburgh EH4 2XU (UK)
E-Mail Joanna.Wardlaw@ed.ac.uk

cally showed no difference between lacunar stroke and non-stroke. Compared to other ischaemic stroke subtypes, there was no difference (CRP) or insufficient or conflicting data (TNF-α) to lacunar stroke. IL-6 was significantly lower [atherothrombotic SMD –0.37 (–0.63, –0.10); cardioembolic SMD –0.52 (–0.82, –0.22)] in lacunar stroke acutely, with insufficient data chronically. ***Key Messages:*** Lacunar stroke is an important stroke subtype. More studies comparing lacunar stroke to non-lacunar stroke specifically, rather than to non-stroke controls, are needed. Prospective studies with measurements taken well after the acute event are more likely to be helpful in determining pathogenesis. The available data in this review were limited and do not exclude the possibility that peripheral inflammatory processes including endothelial dysfunction are associated with lacunar stroke and cerebral small vessel disease.

Introduction

Lacunar stroke is an important stroke subtype, accounting for one quarter of ischaemic strokes. Its aetiology probably differs from other stroke subtypes, usually being the symptomatic manifestation of small vessel disease rather than large vessel atheroma or cardioembolism.

A previous review [1] of symptomatic lacunar stroke versus mainly non-stroke controls suggests a pathogenic role for endothelial dysfunction but this could simply reflect having an ischaemic stroke in general [2]. Another review [3] found C-reactive protein (CRP), P-selectin and homocysteine to differ significantly between ischaemic stroke (in general) and non-stroke controls, but did not assess levels of blood markers between ischaemic stroke subtypes.

We sought to clarify if differences exist in blood markers between lacunar stroke and other ischaemic stroke subtypes by reviewing the literature for studies measuring coagulation, fibrinolysis, endothelial dysfunction and inflammation. We sought to disentangle the acute phase response by splitting the analysis on timing of the blood draw in relation to the stroke.

Methods

This review has been prepared in accordance with The PRISMA statement [4]. We extracted data and conducted the meta-analysis in accordance with MOOSE [5], which was modified for our needs using 3 reporting standards [6–8].

Search Strategy

We used OVID to search MEDLINE (from 1966) and Embase (from 1980) on July 15th, 2012 using a strategy (see Appendix) developed with advice from the Cochrane Stroke Group (http://stroke.cochrane.org/).

Inclusion and Exclusion

We included English language studies published in full measuring blood markers in plasma or serum as follows: tissue plasminogen activator (tPA) as an indicator of fibrinolytic and thrombotic state; plasminogen activator inhibitor (PAI) as an inhibitor of tPA; fibrinogen as a measure of coagulation; D-dimer as a measure of fibrinolysis; homocysteine as a marker of endothelial toxicity; von Willebrand factor (vWF) as a marker of endothelial damage; E-selectin, P-selectin, intercellular adhesion molecule-1 (ICAM) and vascular cellular adhesion molecule-1 (VCAM) as markers of endothelial activation and CRP, interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF-α) as markers of inflammation. We excluded studies that did not provide information on blood markers in lacunar stroke and a control group.

Data Extracted

We extracted data on study population (sample size, age, sex, co-morbidities, current medications); study design; control group (including details on matching for age, sex and covariates where given); blinding of stroke assessor to blood markers and assay investigator to stroke diagnosis; blood markers assessed; time to all blood draws; whether the authors gave detailed methods on how samples were obtained, stored and processed, and results of blood marker levels (both measures of average and spread where given). We recorded method of stroke diagnosis, criteria/system used for stroke subtyping, details of imaging, definition of lacunar stroke and the grade of clinician involved in the stroke diagnosis. We contacted one author [9] to clarify time of blood draw. Some studies drew blood at multiple time points. We recorded data at multiple time points where a relevant comparator was also available. We avoided duplicate publications.

Definition of Lacunar Stroke

Our gold standard definition of lacunar stroke was all of the following: reference to one of the classical clinical lacunar syndromes; no evidence of cortical dysfunction; imaging (including noting a normal scan does not exclude a lacunar diagnosis), and mention of expertise of person who subtyped the stroke. We relied on the clinical stroke definitions used in the primary papers but tried to harmonize these to a clinical stroke syndrome with support from imaging where available [10].

Meta-Analysis

We used the Review Manager 5 software (the Cochrane Collaboration) to calculate standardized mean differences (SMD, where data were in a suitable format) using the inverse variance method and a fixed effects model with 95% confidence intervals (CI). Our forest plots compare lacunar to non-stroke or to non-lacunar stroke (atherothrombotic stroke and/or cardioembolic stroke, as appropriate). Studies [11–13] reporting a geometric mean with 95% CI were converted to standard deviations (SD) using methods described in the Cochrane Handbook [14].

Not all studies could be meta-analysed due to heterogeneity of reporting, e.g. studies reporting medians with either interquartile

ranges or minimum and maximum values, as we could not assume normality of distributions. Consequently, tPA, PAI, E-selectin, P-selectin, ICAM, VCAM, CRP and TNF-α were reviewed solely by summary of individual study data. Where studies provided data at more than one acute time point, we meta-analysed only the first time point as this most often corresponded with data for non-stroke comparators. In order to see if an acute phase response affected the results we split our analysis into 'acute' and 'chronic' (bloods drawn up to and after 21 days of stroke, respectively).

Results

We identified 1,468 full papers. In all, 1,389 titles were excluded following a survey of titles and abstracts, leaving 79 for reading. Of these 10 were excluded (unable to translate) and 32 did not meet inclusion criteria (duplicates, asymptomatic subjects, non-relevant blood markers and no control group). Hand-searching identified a further 5 papers. Therefore, 42 papers were eligible, including 4,816 ischaemic strokes, of which 2,196 were lacunar and 2,500 non-stroke controls (see online suppl. fig. 1 and suppl. table 1; for all online suppl. material, see www.karger.com/doi/10.1159/000356789). In a further 4 papers [15–18] blood was collected prior to stroke. These studies are reported separately (see online suppl. material).

Critical Appraisal of Included Studies

Over 50% of studies (22/42) used TOAST [19] to subtype ischaemic stroke. Just 4 studies [11, 20–22] (fewer than 10%) met our gold standard definition of lacunar stroke. More than half of the studies (57%) reported a 'minimal' definition of lacunar stroke; 1 study [23] failed to define lacunar stroke. Most studies recruited patients consecutively (31/42); 1 study recruited non-consecutively [20] and 10/42 did not report on recruitment. Two thirds of studies reported excluding cases based on co-existing disease such as concurrent infection, cancer, inflammatory disease and renal failure. One third of studies did not report on exclusion criteria. Most studies reported matching controls by age and sex to cases (24/42). Some studies matched age only (6/42) and 1 study matched sex only; 3 studies did not report on matching. Matching for co-morbidities varied from study to study. Less than 20% of studies (8/42) reported blinding of stroke assessor to blood marker values. Fewer still reported blinding of laboratory staff to stroke data (see online suppl. tables 3 and 4 for critical appraisal of included studies).

Plasma Markers

Coagulation/Fibrinolysis

Tissue Plasminogen Activator. Here, we included 5 studies [24–28] (251 lacunar strokes) of which 1 study [26] contributed 50% of the data. There were insufficient data in an appropriate format for meta-analysis. Individual studies suggest tPA was significantly higher in lacunar stroke versus non-stroke controls, both acutely and chronically. Meanwhile, tPA does not appear to differ between lacunar stroke and other stroke subtypes, either acutely or chronically.

Plasminogen Activator Inhibitor. A total of 7 studies were included [24–30] (336 lacunar strokes) but available data did not permit meta-analysis. Jood et al. [26] report levels of PAI significantly higher in lacunar stroke versus non-stroke controls acutely and chronically. They also report lower PAI in lacunar stroke versus atherothrombotic and cardioembolic stroke acutely (significance not given) but not chronically [26]; 4 other studies report no difference between lacunar and non-lacunar stroke acutely [24, 27–29].

Fibrinogen. We included 9 studies [20, 23, 25, 31–36] (622 lacunar strokes) of which 6 permitted a meta-analysis (fig. 1). Fibrinogen showed no difference in lacunar stroke versus non-stroke controls acutely but was significantly higher in lacunar stroke chronically. Fibrinogen was significantly lower in lacunar stroke versus other ischaemic stroke subtypes, both acutely [atherothrombotic SMD −0.37 (95% CI −0.51, −0.22); cardioembolic SMD −0.83 (−1.15, −0.51)] and chronically (fig. 1), although the chronic data comprised only 1 study [32]. In 2 [31, 34] of 3 [31, 33, 34] studies that could not be meta-analysed no difference was reported in levels of fibrinogen between lacunar and non-lacunar stroke in the acute phase; the third study [33] did not report on whether its findings were significant.

D-Dimer. Here, 9 studies were included [25, 30, 32, 34, 37–41] (364 lacunar strokes) of which 4 could be meta-analysed (fig. 2). D-dimer was significantly higher in lacunar stroke versus non-stroke controls, both acutely [SMD 1.42 (1.14, 1.69)] and chronically [SMD 3.22 (2.65, 3.78)]. D-dimer was significantly lower in lacunar versus non-lacunar stroke, both atherothrombotic [acute SMD −3.59 (−4.06, −3.12)] and cardioembolic [acute SMD −5.73 (−6.38, −5.09)], acutely and chronically.

The largest (n = 128 lacunar strokes) of the 5 studies [39] that could not be meta-analysed found no difference in D-dimer between lacunar and atherothrombotic stroke acutely (which disagrees with the meta-analysis) and

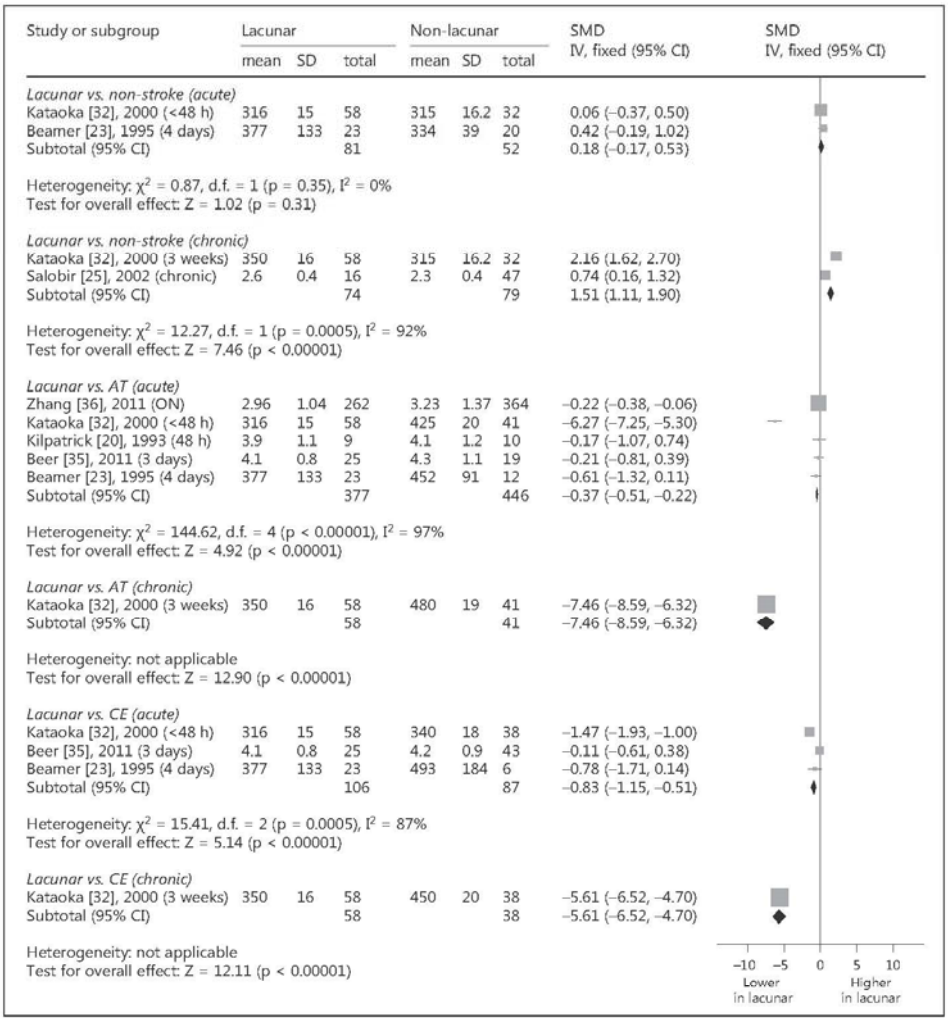


Fig. 1. Forest plot – fibrinogen: SMD of blood markers in lacunar stroke versus non-stroke controls and versus non-lacunar stroke controls at different times after stroke. AT = Atherothrombotic; CE = cardioembolic; ON = on admission.

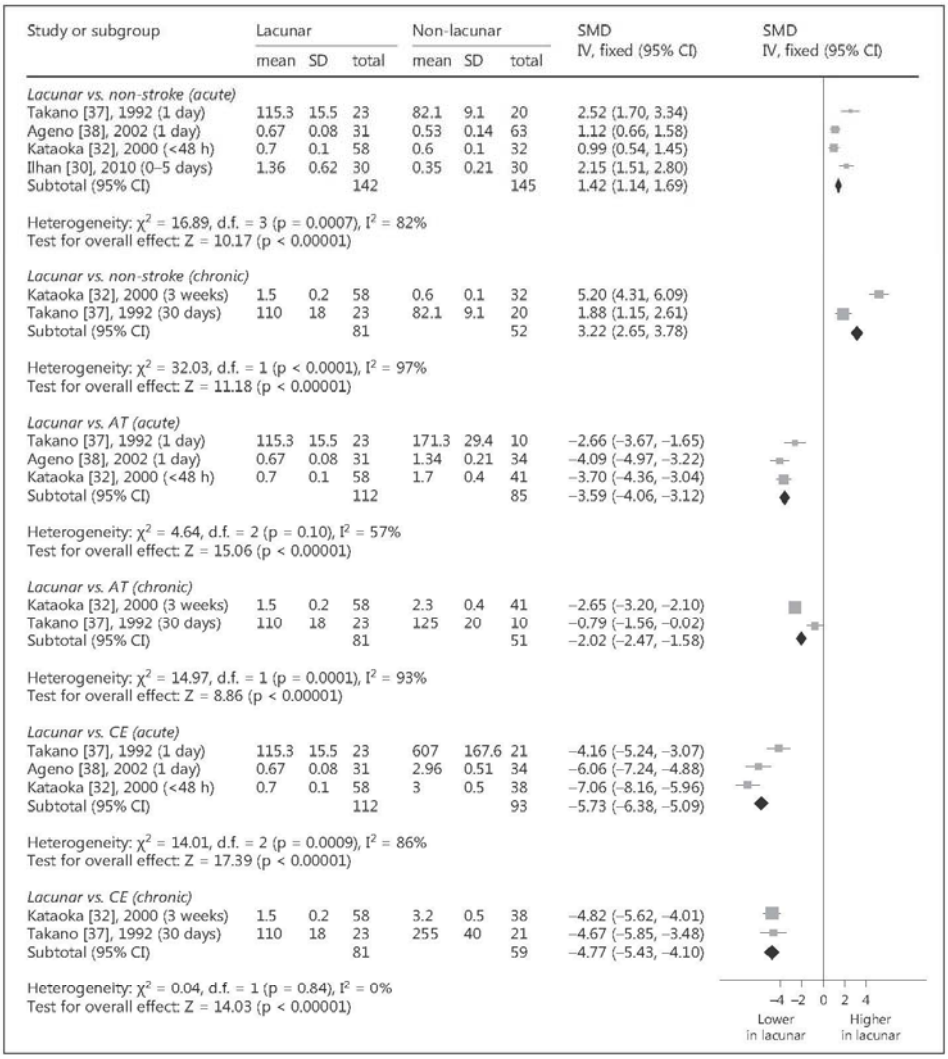


Fig. 2. Forest plot – D-dimer: SMD of blood markers in lacunar stroke versus non-stroke controls and versus non-lacunar stroke controls at different times after stroke. AT = Atherothrombotic; CE = cardioembolic.

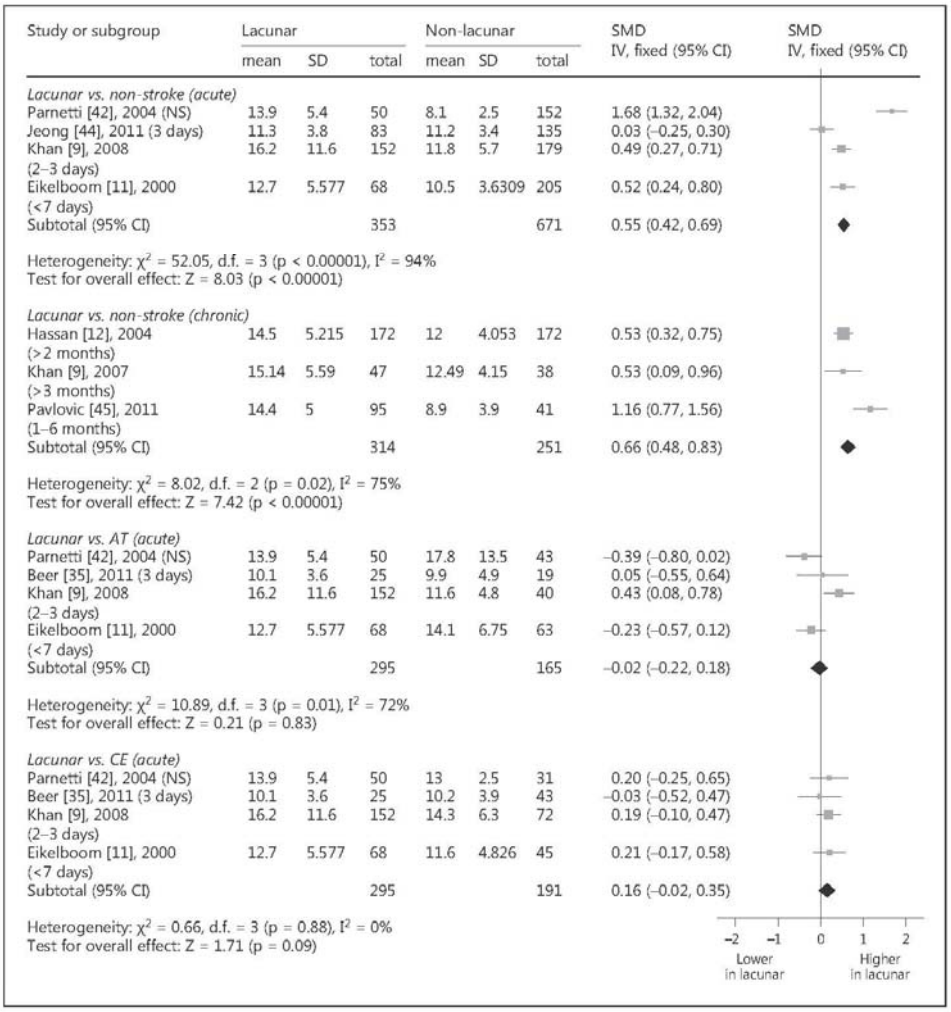


Fig. 3. Forest plot – homocysteine: SMD of blood markers in lacunar stroke versus non-stroke controls and versus non-lacunar stroke controls at different times after stroke. AT = Atherothrombotic; CE = cardioembolic; NS = not stated.

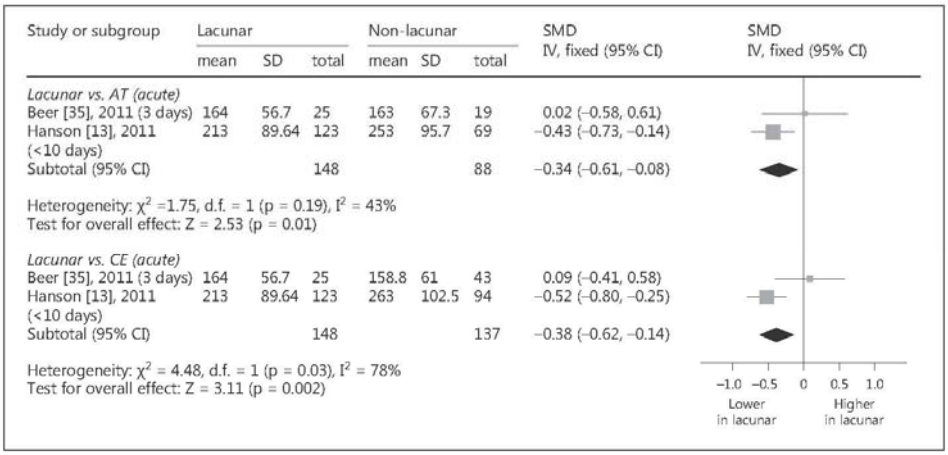


Fig. 4. Forest plot – vWF: SMD of blood markers in lacunar stroke versus non-lacunar stroke controls at different times after stroke. AT = Atherothrombotic; CE = cardioembolic.

found D-dimer significantly lower in lacunar versus cardioembolic stroke acutely (in agreement with the meta-analysis).

Endothelial Activation/Dysfunction

Homocysteine. Overall, we included 9 studies [9, 11, 12, 29, 35, 42–45] (747 lacunar strokes) of which 8 could be meta-analysed (fig. 3). Homocysteine was significantly higher in lacunar stroke versus non-stroke controls, both acutely [SMD 0.55 (0.42, 0.69)] and chronically. Studies comparing lacunar to non-lacunar stroke drew blood acutely only and found no difference [atherothrombotic SMD –0.02 (–0.22, 0.18); cardioembolic SMD 0.16 (–0.02, 0.35)]. In 1 study [29] not included in the meta-analysis no difference was found in homocysteine between lacunar and non-lacunar stroke acutely.

von Willebrand Factor. We included 6 studies [13, 27, 31, 35, 46, 47] (293 lacunar strokes) of which 2 [13, 35] were meta-analysable (fig. 4). vWF was significantly higher in lacunar stroke versus non-stroke controls acutely. vWF was significantly lower in lacunar versus non-lacunar stroke [atherothrombotic SMD –0.34 (–0.61, –0.08); cardioembolic SMD –0.38 (–0.62, –0.14)] acutely. The 4 non-meta-analysable [27, 31, 46, 47] studies show conflicting results (online suppl. table 1).

E-Selectin. Here, 4 studies were included [27, 28, 35, 46] (130 lacunar strokes) but available data did not permit meta-analysis; 1 study [46] found E-selectin significantly higher in lacunar stroke versus non-stroke controls acutely but not at 1 month. Individual study reports suggest no difference between lacunar and non-lacunar stroke.

P-Selectin. A total of 7 studies were included [27, 28, 30, 31, 46, 48, 49] (227 lacunar strokes) but available data did not permit meta-analysis. P-selectin was significantly higher in lacunar versus non-stroke acutely in 2 studies [46, 49] but 1 study [30] found no difference. Another study [48] found P-selectin significantly lower in lacunar versus atherothrombotic stroke acutely but all other studies found no difference or did not report significance levels. Tsai et al. [48] continued to show levels to be significantly lower at 1 month but this difference disappeared at 3 months. Only 1 study [31] found P-selectin significantly lower in lacunar versus cardioembolic stroke acutely; all other studies found no difference or did not report significance levels.

Intercellular Adhesion Molecule-1. We included 5 studies [21, 27, 28, 50, 51] (344 lacunar strokes) but available data did not permit meta-analysis. In 1 study [21] significantly higher ICAM was found in lacunar

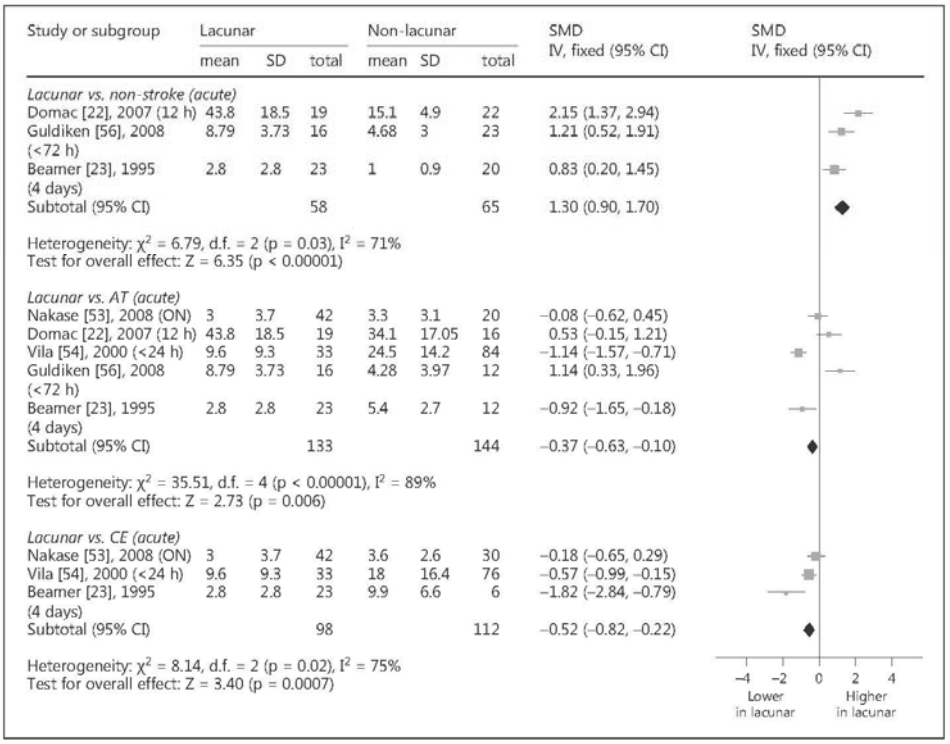


Fig. 5. Forest plot – IL-6: SMD of blood markers in lacunar stroke versus non-stroke controls and versus non-lacunar stroke controls at different times after stroke. AT = Atherothrombotic; CE = cardioembolic; ON = on admission.

stroke versus non-stroke controls acutely; another study [50] found ICAM significantly higher in lacunar versus non-stroke controls chronically. There was no difference between lacunar and other stroke subtypes acutely and no data for lacunar versus non-lacunar stroke chronically.

Vascular Cellular Adhesion Molecule-1. Here, 3 studies were included [27, 28, 51] (121 lacunar strokes) but available data did not permit meta-analysis. Results suggested no difference between lacunar stroke and non-stroke acutely and no difference between lacunar and non-lacunar stroke acutely. No studies measured VCAM chronically.

Inflammation

C-Reactive Protein. We included 8 studies [29, 34, 35, 39, 47, 49, 52, 53] (490 lacunar strokes) but available data did not permit meta-analysis; 2 studies [39, 52] provided just over 50% of the data. CRP was higher in lacunar stroke versus non-stroke acutely in 3 studies [34, 49, 52], significantly so in 2, and the other [34] did not report if the higher value was significant. CRP was significantly lower in lacunar versus atherothrombotic stroke in 1 study [34] acutely; all other studies reported no difference (or did not state significance) between lacunar and non-lacunar stroke, acutely and chronically.

Tumour Necrosis Factor-alpha. Overall, 5 studies were included [21, 22, 27, 47, 53] (252 lacunar strokes) but available data did not permit meta-analysis; 1 study [21] provides 45% of the data. In 2 studies [21, 22] levels of TNF-α were found to be significantly higher in lacunar stroke versus non-stroke controls acutely. TNF-α was significantly lower in 2 [27, 47] and no different in 2 [22, 53] studies reporting on lacunar versus non-lacunar stroke acutely. No studies measured TNF-α chronically.

Interleukin-6. A total of 9 studies were included [21–23, 27, 47, 53–56] (340 lacunar strokes) of which 5 could be meta-analysed (fig. 5). IL-6 was significantly higher in lacunar stroke versus non-stroke acutely [SMD 1.3 (0.9, 1.7)], and significantly lower in lacunar versus non-lacunar stroke [atherothrombotic SMD –0.37 (–0.63, –0.10); cardioembolic SMD –0.52 (–0.82, –0.22)] acutely.

In 1 non-meta-analysable study [21] IL-6 was found to be significantly higher in lacunar stroke versus non-stroke acutely, in agreement with the meta-analysed studies; 2 non-meta-analysable studies [27, 47] found IL-6 significantly lower in lacunar versus non-lacunar stroke acutely, also in agreement with the meta-analysed studies.

The only long-term study [55] found no difference between lacunar stroke and non-stroke controls from a wholly female cohort measured 3.5 years after stroke.

Discussion

This review assessed blood markers of coagulation, fibrinolysis, endothelial dysfunction and inflammation in lacunar stroke versus non-stroke controls and other ischaemic stroke subtypes. While many markers were higher in lacunar stroke than in non-stroke controls, they were mostly lower in lacunar versus non-lacunar stroke. A brief summary follows.

Coagulation/Fibrinolysis

tPA/PAI were significantly higher in lacunar stroke versus non-stroke, acutely and chronically, and did not differ between lacunar and non-lacunar stroke, acutely or chronically.

Fibrinogen did not differ between lacunar stroke and non-stroke acutely, although we only used a single time point from Kataoka et al. [32] (bloods drawn at <24 h). If we consider their second sample (at 7 days) as a further acute measurement, lacunar stroke tends towards being significantly higher than non-stroke. Fibrinogen was significantly higher in lacunar stroke versus non-stroke chronically. Fibrinogen was significantly lower in lacunar versus non-lacunar stroke, acutely and chronically. However, studies excluded from the meta-analysis tended to show no overall difference between lacunar and non-lacunar stroke and chronic data came from a single study.

D-dimer was significantly higher in lacunar stroke versus non-stroke, acutely and chronically, and significantly lower in lacunar versus non-lacunar stroke, acutely and chronically.

Endothelial Dysfunction

Homocysteine was significantly higher in lacunar stroke versus non-stroke, acutely and chronically, but did not differ between lacunar and non-lacunar stroke acutely (but there were no chronic phase studies).

vWF was significantly higher in lacunar stroke versus non-stroke, acutely, with conflicting evidence chronically. vWF was significantly lower in lacunar than non-lacunar stroke acutely (2 studies), with conflicting but non-meta-analysable evidence in other studies both acutely and chronically.

E-selectin was significantly higher in lacunar stroke versus non-stroke acutely (only 1 study) but not chronically and did not differ between lacunar and non-lacunar stroke, either acutely or chronically (only 1 study).

P-selectin was significantly higher in lacunar stroke versus non-stroke acutely in some but not all studies, and in the only study that reported a chronic measurement. P-selectin did not differ between lacunar and non-lacunar stroke, either acutely or chronically (only 1 study).

ICAM was significantly higher in lacunar stroke versus non-stroke, acutely and chronically (only 1 study), and did not differ between lacunar and non-lacunar stroke acutely (with no studies chronically).

VCAM did not differ between lacunar stroke and non-stroke nor between lacunar and non-lacunar stroke acutely. There were no studies chronically.

Inflammation

CRP was significantly higher in lacunar stroke versus non-stroke, acutely and chronically (only 1 study) and did not differ between lacunar and non-lacunar stroke acutely or chronically (only 1 study).

TNF-α was significantly higher in lacunar stroke versus non-stroke acutely with no studies chronically. There was conflicting evidence on levels of TNF-α in lacunar versus non-lacunar stroke acutely with no studies chronically.

IL-6 was significantly higher in lacunar stroke versus non-stroke acutely but did not differ chronically. IL-6 was

significantly lower in lacunar versus non-lacunar stroke acutely, but there were no chronic phase studies.

This suggests that plasma marker elevation in lacunar stroke is likely to reflect the process of having a stroke rather than that systemic inflammation or endothelial dysfunction is specific to lacunar stroke. The available data were limited and do not exclude the possibility that peripheral inflammatory or endothelial dysfunction processes are associated with lacunar stroke specifically.

There were limitations to the studies. Most were small, with varying methods and an inconsistent definition of 'lacunar stroke', as highlighted previously [57]. Papers reviewed used the term lacunar stroke to reflect a clinical entity, i.e. clinical presentation with a stroke, but definitions varied and we refer readers to the new neuroimaging standards [10]. We were not able to differentiate different mechanisms of lacunar stroke. Most lacunar strokes are due to recent small subcortical infarcts, and most of these relate to intrinsic small vessel disease. However, they also arise from atherothromboembolism (large artery) or cardioembolism in a small proportion of patients and it was not possible to differentiate these cases.

There was heterogeneity across several aspects of the methods. Many used TOAST [19] but as this uses risk factors to categorize patients it potentially introduces classification bias. A patient with an unclear diagnosis of lacunar stroke but concurrent hypertension or diabetes might (rightly or wrongly) be classified as 'lacunar' using this system, although hypertension and diabetes were equally prevalent risk factors between ischaemic stroke subtypes in 21,980 stroke patients when subtypes were classified without risk factors [58]. Several did not report on whether their findings achieved statistical significance; in the absence of an explicit statement, we report this as 'not stated'. In some studies blood was drawn after overnight fasting, whereas in others non-fasting blood was collected. Studies used different units of measurement and assay methods. None reported on whether the patients had recent infection or neutrophilia, or if these patients were excluded. Timing of blood draw in relation to stroke varied but is important to account for each marker's individual 'response curve' which changes over time. Fassbender et al. [59] found levels of IL-6 to rise rapidly following onset of ischaemic stroke, reaching a plateau at 10 h until 3 days before returning to normal by day 7. They did not subtype stroke and hence their study was not included in this review. Between-study heterogeneity on time to blood draw complicates subsequent analysis, although meta-analyses use within-study data and so will have minimized any effect of between-study variation.

Our review had limitations. We did not study markers in cerebrospinal fluid. We did not review the association of marker levels with lesion size or clinical outcome as data were sparse. Ahmad et al. [60] found markers of neuronal damage correlated with infarct size, which might explain why marker levels in non-lacunar stroke were frequently higher than in lacunar stroke in the acute phase. We were not able to analyse differences between groups reported as top versus bottom quantiles.

Our review had strengths, including assessment of differences between stroke subtypes, quality assessment of included studies, meticulous extraction of data and meta-analysis thereof, wherever suitable data were available. Previous reviews compared lacunar stroke to non-stroke controls only and therefore did not distinguish lacunar stroke specifically from stroke in general.

To determine if there is a difference in coagulation, fibrinolysis, endothelial dysfunction and inflammation in lacunar versus other stroke subtypes requires a large prospective study of blood markers in accurately phenotyped patients with lacunar versus non-lacunar stroke classified using non-risk-factor-based definitions. Future studies should clearly define and diagnose lacunar stroke, avoid subtyping stroke using risk factor-based classifications, explicitly report negative findings and significance levels, and obtain blood several weeks post-stroke to avoid confounding from an acute phase response.

Appendix

Search Strategy.

(1) brain ischemia/ or brain infarction/ or brain stem infarctions/ or cerebral infarction/ or hypoxia-ischemia, brain/ or stroke/
(2) (isch?emi$ adj6 (stroke$ or apoplex$ or cerebral vasc$ or cerebrovasc$ or cva or attack$)).tw.
(3) ((brain or cerebr$ or cerebell$ or vertebrobasil$ or hemispher$ or intracran$ or intracerebral or infratentorial or supratentorial or middle cerebr$ or mca$ or anterior circulation) adj5 (isch?emi$ or infarct$ or thrombo$ or emboli$ or occlus$ or hypoxi$)).tw.
(4) 1 or 2 or 3
(5) (lacun$ or small vessel$ or small infarct$ or microinfarct$ or subcortical lesion$ or subcortical infarct$ or microvascular$ or microcirculation$).tw.
(6) 4 and 5
(7) blood-brain barrier/ or endothel$, vascular/ or tunica intima/ or microcirculation/
(8) (endotheli$ adj5 (function$ or dysfunction$ or impairment$)).tw.
(9) (endogenous tissue plasminogen activator or endogenous tPA).tw

(10) thrombosis.tw
(11) fibrinogen.tw
(12) fibrinolysis.tw
(13) homocysteine.tw
(14) (ICAM or Intra cellular adhesion molecule).tw
(15) (VCAM or Vascular Cell Adhesion Molecule).tw
(16) (IL6 or Interleukin 6).tw
(17) (CRP or C reactive protein).tw
(18) von Willebrand factor.tw
(19) plasminogen activator inhibitor.tw
(20) selectin$.tw
(21) D-dimer.tw
(22) (TNF or TNF-a or TNF-alpha or TNF-α or tumo?r necrosis factor alpha).tw
(23) or/7–22
(24) 6 and 23
(25) limit 24 to humans

Acknowledgements

The authors acknowledge Dr. Stephen Makin for assistance in collecting some of the data. Stewart Wiseman is supported by a Principal's Career Development PhD Scholarship from the University of Edinburgh.

Disclosure Statement

None.

References

1 Knottnerus IL, Ten Cate H, Lodder J, Kessels F, Van Oostenbrugge RJ: Endothelial dysfunction in lacunar stroke: a systematic review. Cerebrovasc Dis 2009;27:519–526.
2 Stevenson SF, Doubal FN, Shuler K, Wardlaw JM: A systematic review of dynamic cerebral and peripheral endothelial function in lacunar stroke versus controls. Stroke 2010; 41:e434–e442.
3 Hasan N, McColgan P, Bentley P, Edwards RJ, Sharma P: Towards the identification of blood biomarkers for acute stroke in humans: a comprehensive systematic review. Br J Clin Pharmacol 2012;74:230–240.
4 Moher D, Liberati A, Tetzlaff J, Altman DG: Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med 2009;6:e1000097.
5 Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, Moher D, Becker BJ, Sipe TA, Thacker SB: Meta-analysis of observational studies. JAMA 2000;283:2008–2012.
6 Mischak H, Allmaier G, Apweiler R, Attwood T, Baumann M, Benigni A, Bennett SE, Bischoff R, Bongcam-Rudloff E, Capasso G, Coon JJ, D'Haese P, Dominiczak AF, Dakna M, Dihazi H, et al: Recommendations for biomarker identification and qualification in clinical proteomics. Sci Transl Med 2010;2: 46ps42.
7 McShane LM, Altman DG, Sauerbrei W, Taube SE, Gion M, Clark GM: Reporting Recommendations for Tumour Marker Prognostic Studies (REMARK). Eur J Cancer 2005;41: 1690–1696.
8 Von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP: The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. Rev Esp Salud Publica 2007;82:251–259.
9 Khan U, Crossley C, Kalra L, Rudd A, Wolfe CDA, Collinson P, Markus HS: Homocysteine and its relationship to stroke subtypes in a UK black population: the south London ethnicity and stroke study. Stroke 2008;39:2943–2949.
10 Wardlaw JM, Smith EE, Biessels GJ, Cordonnier C, Fazekas F, Frayne R, Lindley RI, O'Brien JT, Barkhof F, Benavente OR, Black SE, Brayne C, Breteler M, Chabriat H, Decarli C, et al: Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration. Lancet Neurol 2013;12:822–838.
11 Eikelboom JW, Hankey GJ, Anand SS, Lofthouse E, Staples N, Baker RI: Association between high homocyst(e)ine and ischemic stroke due to large- and small-artery disease but not other etiologic subtypes of ischemic stroke. Stroke 2000;31:1069–1075.
12 Hassan A, Hunt BJ, O'Sullivan M, Bell R, D'Souza R, Jeffery S, Bamford JM, Markus HS: Homocysteine is a risk factor for cerebral small vessel disease, acting via endothelial dysfunction. Brain 2004;127:212–219.
13 Hanson E, Jood K, Karlsson S, Nilsson S, Blomstrand C, Jern C: Plasma levels of von Willebrand factor in the etiologic subtypes of ischemic stroke. J Thromb Haemost 2011;9: 275–281.
14 Higgins J, Greens S: Cochrane Handbook for Systematic Reviews of Interventions, version 5.1.0 (updated March 2011). The Cochrane Collaboration, 2011 (available from www.cochrane-handbook.org).
15 Iso H, Moriyama Y, Sato S, Kitamura A, Tanigawa T, Yamagishi K, Imano H, Ohira T, Okamura T, Naito Y, Shimamoto T: Serum total homocysteine concentrations and risk of stroke and its subtypes in Japanese. Circulation 2004;109:2766–2772.
16 Ohira T, Shahar E, Chambless LE, Rosamond WD, Mosley TH, Folsom AR: Risk factors for ischemic stroke subtypes: the Atherosclerosis Risk in Communities study. Stroke 2006;37: 2493–2498.
17 Andersson J, Johansson L, Ladenvall P, Wiklund P-G, Stegmayr B, Jern C, Boman K: C-reactive protein is a determinant of first-ever stroke: prospective nested case-referent study. Cerebrovasc Dis 2009;27:544–551.
18 Chei C-L, Yamagishi K, Kitamura A, Kiyama M, Imano H, Ohira T, Tanigawa T, Sankai T, Ishikawa Y, Sato S, Iso H: C-reactive protein levels and risk of stroke and its subtype in Japanese: the Circulatory Risk in Communities Study (CIRCS). Atherosclerosis 2011;217:187–193.
19 Adams HP, Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, Marsh EE: Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. Stroke 1993;24:35–41.
20 Kilpatrick TJ, Matkovic Z, Davis SM, McGrath CM, Dauer RJ: Hematologic abnormalities occur in both cortical and lacunar infarction. Stroke 1993;24:1945–1950.
21 Castellanos M, Castillo J, Garcia MM, Leira R, Serena J, Chamorro A, Davalos A: Inflammation-mediated damage in progressing lacunar infarctions: a potential therapeutic target. Stroke 2002;33:982–987.
22 Domac FM, Somay G, Misirli H, Erenoglu NY: Tumor necrosis factor alpha serum levels and inflammatory response in acute ischemic stroke. Neurosciences 2007;12:25–30.
23 Beamer NB, Coull BM, Clark WM, Hazel JS, Silberger JR: Interleukin-6 and interleukin-1 antagonist in acute stroke. Ann Neurol 1995; 37:800–805.

24 Lindgren A, Lindoff C, Norrving B, Astedt B, Johansson BB: Tissue plasminogen activator and plasminogen activator inhibitor-1 in stroke patients. Stroke 1996;27:1066–1071.
25 Salobir B, Sabovic M, Peternel P, Stegnar M, Grad A: Classic risk factors, hypercoagulability and migraine in young women with cerebral lacunar infarctions. Acta Neurol Scand 2002;105:189–195.
26 Jood K, Ladenvall P, Tjärnlund-Wolf A, Ladenvall C, Andersson M, Nilsson S, Blomstrand C, Jern C: Fibrinolytic gene polymorphism and ischemic stroke. Stroke 2005;36:2077–2081.
27 Tuttolomondo A, Di Sciacca R, Di Raimondo D, Serio A, D'Aguanno G, La Placa S, Pecoraro R, Arnao V, Marino L, Monaco S, Natalè E, Licata G, Pinto A: Plasma levels of inflammatory and thrombotic/fibrinolytic markers in acute ischemic strokes: relationship with TOAST subtype, outcome and infarct site. J Neuroimmunol 2009;215:84–89.
28 Tuttolomondo A, Pinto A, Corrao S, Di Raimondo D, Fernandez P, Di Sciacca R, Arnao V, Licata G: Immuno-inflammatory and thrombotic/fibrinolytic variables associated with acute ischemic stroke diagnosis. Atherosclerosis 2009;203:503–508.
29 Yokokawa H, Goto A, Terui K, Funami Y, Watanabe K, Yasumura S: Prevalence of metabolic syndrome and serum marker levels in patients with four subtypes of cerebral infarction in Japan. J Clin Neurosci 2008;15:769–773.
30 Ilhan D, Ozbabalik D, Gulcan E, Ozdemir O, Gülbaçs Z: Evaluation of platelet activation, coagulation, and fibrinolytic activation in patients with symptomatic lacunar stroke. Neurologist 2010;16:188–191.
31 Bath PMW, Blann A, Smith N, Butterworth RJ: Von Willebrand factor, P-selectin and fibrinogen levels in patients with acute ischaemic and haemorrhagic stroke, and their relationship with stroke sub-type and functional outcome. Platelets 1998;9:155–159.
32 Kataoka S, Hirose G, Hori A, Shirakawa T, Saigan T: Activation of thrombosis and fibrinolysis following brain infarction. J Neurol Sci 2000;181:82–88.
33 Jood K, Danielson J, Ladenvall C, Blomstrand C, Jern C: Fibrinogen gene variation and ischemic stroke. J Thromb Haemost 2008;6:897–904.
34 Alvarez-Perez FJ, Castelo-Branco M, Alvarez-Sabin J: Usefulness of measurement of fibrinogen, D-dimer, D-dimer/fibrinogen ratio, C-reactive protein and erythrocyte sedimentation rate to assess the pathophysiology and mechanism of ischaemic stroke. J Neurol Neurosurg Psychiatry 2011;82:986–992.
35 Beer C, Blacker D, Hankey GJ, Puddey IB: Association of clinical and aetiologic subtype of acute ischaemic stroke and inflammation, oxidative stress and vascular function: a cross-sectional observational study. Med Sci Monit 2011;17:467–473.
36 Zhang B, Zhang W, Li X, Pu S, Yin J, Yang N, Yi Y, Gao Q, Gao C: Admission markers predict lacunar and non-lacunar stroke in young patients. Thromb Res 2011;128:14–17.
37 Takano K, Yamaguchi T, Uchida K: Markers of a hypercoagulable state following acute ischemic stroke. Stroke 1992;23:194–198.
38 Ageno W, Finazzi S, Steidl L, Biotti MG, Mera V, Melzi D'Eril G, Venco A: Plasma measurement of D-dimer levels for the early diagnosis of ischemic stroke subtypes. Arch Intern Med 2002;162:2589–2593.
39 Montaner J, Perea-Gainza M, Delgado P, Ribó M, Chacón P, Rosell A, Quintana M, Palacios ME, Molina CA, Alvarez-Sabín J: Etiologic diagnosis of ischemic stroke subtypes with plasma biomarkers. Stroke 2008;39:2280–2287.
40 Brouns R, Van den Bossche J, De Surgeloose D, Sheorajpanday R, De Deyn PP: Clinical and biochemical diagnosis of small-vessel disease in acute ischemic stroke. J Neurol Sci 2009;285:185–190.
41 Isenegger J, Meier N, Lämmle B, Alberio L, Fischer U, Nedeltchev K, Gralla J, Kohler H-P, Mattle HP, Arnold M: D-dimers predict stroke subtype when assessed early. Cerebrovasc Dis 2010;29:82–86.
42 Parnetti L, Caso V, Santucci a A, Corea F, Lanari A, Floridi A, Conte C, Bottiglieri T: Mild hyperhomocysteinemia is a risk factor in all etiological subtypes of stroke. Neurol Sci 2004;25:13–17.
43 Khan U, Hassan A, Vallance P, Markus HS: Asymmetric dimethylarginine in cerebral small vessel disease. Stroke 2007;38:411–413.
44 Jeong S-K, Kim D-H, Cho YI: Homocysteine and pulsatility index in lacunar infarction. Clin Neurol Neurosurg 2011;113:459–463.
45 Pavlovic AM, Pekmezovic T, Obrenovic R, Novakovic I, Tomic G, Mijajlovic M, Sternic N: Increased total homocysteine level is associated with clinical status and severity of white matter changes in symptomatic patients with subcortical small vessel disease. Clin Neurol Neurosurg 2011;113:711–715.
46 Kozuka K, Kohriyama T, Nomura E, Ikeda J, Kajikawa H, Nakamura S: Endothelial markers and adhesion molecules in acute ischemic stroke – sequential change and differences in stroke subtype. Atherosclerosis 2002;161:161–168.
47 Licata G, Tuttolomondo A, Di Raimondo D, Corrao S, Di Sciacca R, Pinto A: Immuno-inflammatory activation in acute cardio-embolic strokes in comparison with other subtypes of ischaemic stroke. Thromb Haemost 2009:929–937.
48 Tsai N-W, Chang W-N, Shaw C-F, Jan C-R, Chang H-W, Huang C-R, Chen S-D, Chuang Y-C, Lee L-H, Wang H-C, Lee T-H, Lu C-H: Levels and value of platelet activation markers in different subtypes of acute non-cardioembolic ischemic stroke. Thromb Res 2009;124:213–218.
49 Turgut B, Turgut N, Celik Y, Tekgündüz E, Pamuk GE, Demir M: Differences in platelet-leukocyte aggregates among subtypes of acute cerebral ischemia. J Neurol Sci 2011;305:126–130.
50 Hassan A, Hunt BJ, O'Sullivan M, Parmar K, Bamford JM, Briley D, Brown MM, Thomas DJ, Markus HS: Markers of endothelial dysfunction in lacunar infarction and ischaemic leukoaraiosis. Brain 2003;126:424–432.
51 Supanc V, Biloglav Z, Kes VB, Demarin V: Role of cell adhesion molecules in acute ischemic stroke. Ann Saudi Med 2011;31:365–370.
52 Ladenvall C, Jood K, Blomstrand C, Nilsson S, Jern C, Ladenvall P: Serum C-reactive protein concentration and genotype in relation to ischemic stroke subtype. Stroke 2006;37:2018–2023.
53 Nakase T, Yamazaki T, Ogura N, Suzuki A, Nagata K: The impact of inflammation on the pathogenesis and prognosis of ischemic stroke. J Neurol Sci 2008;271:104–109.
54 Vila N, Castillo J, Davalos A, Chamorro A: Proinflammatory cytokines and early neurological worsening in ischemic stroke. Stroke 2000;31:2325–2329.
55 Salobir B, Sabovic M: Possible vascular-bed-specific role of interleukin-6 in young women with a history of myocardial infarction, lacunar cerebral infarction and deep vein thrombosis. Cytokine 2004;25:265–272.
56 Guldiken B, Guldiken S, Turgut B, Turgut N, Demir M, Celik Y, Arikan E, Tugrul A: The roles of oxidized low-density lipoprotein and interleukin-6 levels in acute atherothrombotic and lacunar ischemic stroke. Angiology 2008;59:224–229.
57 Potter GM, Marlborough FJ, Wardlaw JM: Wide variation in definition, detection, and description of lacunar lesions on imaging. Stroke 2011;42:359–366.
58 Jackson C, Sudlow C: Are lacunar strokes really different? A systematic review of differences in risk factor profiles between lacunar and nonlacunar infarcts. Stroke 2005;36:891–901.
59 Fassbender K, Rossol S, Kammer T, Daffertshofer M, Wirth S, Dollman M, Hennerici M: Proinflammatory cytokines in serum of patients with acute cerebral ischemia: kinetics of secretion and relation to the extent of brain damage and outcome of disease. J Neurol Sci 1994;122:135–139.
60 Ahmad O, Wardlaw J, Whiteley WN: Correlation of levels of neuronal and glial markers with radiological measures of infarct volume in ischaemic stroke: a systematic review. Cerebrovasc Dis 2012;33:47–54.

"Plasma biomarkers of inflammation, endothelial function and haemostasis in cerebral small vessel disease" (see Chapter 3). Reproduced with permission from the publisher S. Karger AG.

Original Paper

Cerebrovascular Diseases

Cerebrovasc Dis 2015;40:157–164
DOI: 10.1159/000438494

Received: April 20, 2015
Accepted: July 6, 2015
Published online: August 8, 2015

Plasma Biomarkers of Inflammation, Endothelial Function and Hemostasis in Cerebral Small Vessel Disease

Stewart J. Wiseman[a] Fergus N. Doubal[a] Francesca M. Chappell[a]
Maria C. Valdés-Hernández[a] Xin Wang[a] Ann Rumley[b] Gordon D.O. Lowe[b]
Martin S. Dennis[a] Joanna M. Wardlaw[a]

[a]Centre for Clinical Brain Sciences, University of Edinburgh, and [b]Institute of Cardiovascular and Medical Sciences, Royal Infirmary, University of Glasgow, Glasgow, UK

Key Words
Biomarker · Endothelium · Inflammation · Stroke · Lacunar

Abstract
Background: The cause of lacunar ischemic stroke, a clinical feature of cerebral small vessel disease (SVD), is largely unknown. Inflammation and endothelial dysfunction have been implicated. Plasma biomarkers could provide mechanistic insights but current data are conflicting. White matter hyperintensities (WMHs) are an important imaging biomarker of SVD. It is unknown if plasma biomarkers add predictive capacity beyond age and vascular risk factors in explaining WMH. ***Methods:*** We prospectively recruited patients presenting with non-disabling ischemic stroke, classifying them clinically and with the help of MRI as lacunar or cortical. We measured biomarkers of inflammation, endothelial dysfunction and hemostasis for >1 month after stroke and compared biomarker levels between stroke subtypes. We quantitatively calculated WMH. We used multiple linear regression analysis to model WMH as a function of age, sex, hypertension and smoking (the baseline model). We fitted exploratory models using plasma biomarkers as predictor variables to assess model improvement over baseline. ***Results:*** We recruited 125 patients. The lacunar group (n = 65) had lower tissue plasminogen activator (t-PA) levels in unadjusted (7.39 vs. 8.59 ng/ml, p = 0.029) and adjusted (p = 0.035) analyses compared with the cortical group (n = 60). There were no significant differences in the other plasma biomarkers. The results for t-PA were consistent with an updated meta-analysis, although the effect remains non-significant (standardized mean difference –0.08 (95% CI –0.25 to 0.09)). The baseline regression model explained 29% of the variance in quantitative WMH (R^2 0.289). Inflammatory biomarkers showed minor improvement over baseline (R^2 0.291), but the other plasma biomarkers did not improve the baseline model. ***Conclusion:*** Plasma t-PA levels appear to differ between lacunar and cortical stroke subtypes, late after stroke, independent of age, sex and vascular risk factors and may reflect endothelial dysfunction. Except for a minor additional predictive effect of inflammatory markers, plasma biomarkers do not relate to WMH severity in this small stroke population.

S.J.W. and F.N.D. are joint first authors.

KARGER 125

E-Mail karger@karger.com
www.karger.com/ced

Joanna M. Wardlaw
CCBS, Chancellor's Building, Royal Infirmary of Edinburgh
49 Little France Crescent
Edinburgh, EH16 4SB (UK)
E-Mail Joanna.Wardlaw@ed.ac.uk

Introduction

The cause of lacunar ischemic stroke, a clinical feature of cerebral small vessel disease (SVD), is largely unknown although systemic inflammation, endothelial dysfunction, failure of the blood–brain barrier or occlusive microthrombus have been implicated [1–5].

Plasma biomarkers of inflammation, endothelial dysfunction and hemostasis may provide mechanistic insights, although if measured too soon after stroke they might simply reflect the acute effects of stroke rather than background pathway activity.

The MRI features of SVD include white matter hyperintensities (WMHs) and are associated with ischemic and hemorrhagic stroke and dementia [6]. WMH predict an increased risk of stroke [6] and are associated with poor functional outcomes following stroke [7]. Plasma biomarkers may predict outcome after stroke [8] and could have a role in the management of stroke patients [9].

The relationship between plasma biomarkers and imaging biomarkers of SVD is not fully understood. Systematic reviews [2, 10, 11] are impeded by between-study heterogeneity and they differ in their conclusions. Generally, plasma biomarkers are raised in lacunar stroke compared with non-stroke healthy controls (used in most studies) but differences here are unsurprising, especially in the acute phase of stroke. The situation is less clear when lacunar stroke is compared to other ischemic stroke subtypes. In a systematic review and meta-analysis [10], we found differences in levels of fibrinogen, D-dimer, von Willebrand factor (vWF) and interleukin-6 (IL-6) between lacunar and non-lacunar stroke, and no difference or conflicting evidence for other biomarkers.

In 2 large population studies of subjects without stroke, higher inflammatory biomarkers were independently associated with higher WMH volumes [1, 12] but not in 3 other studies [13–15]. Biomarkers of endothelial activation were associated with WMH in a cross-sectional analysis [16] and with WMH progression [4]. Flow-mediated dilatation studies have shown the presence of endothelial dysfunction in lacunar stroke patients compared with non-stroke controls [17].

Prior stroke studies often take the plasma samples too early making it difficult to isolate underlying trends independent from an acute phase response. Few studies assessed a range of biomarkers simultaneously in one population.

The purpose of this study was (1) to determine if there were differences in levels of plasma biomarkers of (a) inflammation, (b) endothelial dysfunction or (c) hemostasis between lacunar and cortical stroke subtypes, well after the acute event, as representative of 3 potential SVD mechanisms, adjusted for age and major vascular risk factors; (2) to update our meta-analysis and place current findings into context and (3) to assess the association between the 3 plasma biomarker groups and WMH, irrespective of stroke subtype.

Methods

Our definition of SVD is in accordance with the STRIVE neuroimaging reporting guidelines [18].

Patients

We prospectively recruited patients, as consecutively as possible, who presented with ischemic stroke of lacunar or mild (i.e. non-disabling) cortical subtype seen at our hospital stroke service, as detailed previously [19]. Patients with cortical stroke acted as controls because they have many similar risk factors, medications and extent of damage due to the acute ischemic stroke to patients with lacunar stroke, thus controlling for potential confounders and allowing us to differentiate findings specific to SVD. We excluded patients with contraindications to MR, hemorrhagic stroke or severe stroke, that is, disabling total anterior circulation stroke. The study was approved by the local research ethics committee (2002/8/64), and all patients gave written informed consent.

Patient Investigations

Patients were assessed at presentation by an experienced stroke physician and all underwent investigations as follows: brain imaging on a 1.5T research MR scanner with a standardized protocol (details available on request), carotid Doppler ultrasound and electrocardiogram. We recorded past medical histories including hypertension, diabetes, hypercholesterolemia and smoking, and measured the blood pressure and blood lipids as per the usual stroke patient assessment.

Stroke Subtype

We assessed stroke severity with the National Institute for Health Stroke Scale (NIHSS) [20] (but did not use NIHSS as selection criteria) and classified the stroke clinical syndrome (lacunar or cortical) according to the Oxfordshire Community Stroke Project [21]. We defined 'lacunar stroke' as per the classical clinical lacunar syndromes (pure motor weakness or sensory loss or both in face and arm, arm and leg or all three, ataxic hemiparesis or clumsy hand dysarthria syndrome). We defined 'mild cortical stroke' as a maximum clinical deficit of either one of the following: weakness or sensory loss in the face, arm or leg, or loss of higher cerebral function (dysphasia or neglect), or weakness in more than one limb in the presence of loss of higher cerebral function (all in keeping with a partial anterior circulation stroke) or a homonymous hemianopia suggestive of occipital cortical infarct (in keeping with a cortical posterior circulation stroke).

We then assessed whether a recent infarct on MR was firstly present and secondly whether it was cortical or lacunar. We based

the final stroke subtype classification on both the clinical and radiological classification. Where the clinical classification differed from the radiological classification, the radiological classification was used – using clinical criteria alone can result in misclassification of infarcts in up to 20% of cases [22]. Where an infarct on imaging was absent, an expert panel with all available information assigned the final stroke subtype.

Plasma Biomarkers

All patients had their blood sampled after a minimum of 1 and maximum of 3 months following the stroke to avoid the acute phase. Samples were spun and frozen for batch analysis, blind to clinical data. We measured markers of inflammation (C-reactive protein (CRP) tumor necrosis factor-alpha (TNF) and IL-6), endothelial activation (vWF and intracellular adhesion molecule-1 (ICAM)) and thrombotic/fibrinolytic activity (fibrinogen, tissue plasminogen activator (t-PA) antigen and D-dimer). Intra- and inter-assay variation on biomarker testing was between 3.3 and 12.5% (online suppl. table 1; for all online suppl. material, see www.karger.com/doi/10.1159/000438494).

Image Analysis

All scans were reviewed by a neuroradiologist for the index infarct and rated for SVD features using standardized scales [23–25] including the Fazekas scale for WMH. A quantitative, volumetric measure of WMH (in mm^3) was also calculated, as described previously [26]. We corrected for head size by dividing the quantitative WMH load by the intracranial volume. We verified the correlation between the visual WMH rating and the quantitative value.

Statistical Analysis

We assessed differences in patient demographics and plasma biomarkers between stroke subtypes using Student's t test, the Mann–Whitney U test and the chi-square test, as appropriate. Quantitative WMH and some plasma biomarkers (CRP, TNF, IL-6 and D-dimer) were not normally distributed; so we log transformed these data.

We explored the association between stroke subtypes and plasma biomarkers with multiple linear regression analysis which allowed us to control for age, sex and vascular risk factors.

We used multiple linear regression to assess the contribution of plasma biomarkers in explaining variance in quantitative WMH volume (n = 98, 27 scans were unavailable for WMH quantification), irrespective of stroke subtype. We repeated the modeling with visually rated WMH, for which the full data set was available (n = 125).

We used standardized units (mean = 0, SD = 1) for all plasma biomarkers in the regression models. The standardized data have no units and are on the same scale, so different biomarkers can be added together into summed variables which reduces the number of predictor variables to help avoid model over-fitting. We verified the correlation between the components of the summed variables. The summed variables were the following: inflammation (INF) (CRP + TNF + IL-6), endothelial dysfunction (END) (vWF + ICAM) and thrombosis (THR) (t-PA + D-dimer + fibrinogen).

We fitted a baseline model with quantitative WMH volume as the outcome measure and age, sex, hypertension and smoking status as the predictor variables. Patients with a past history of tobacco use were classified as non-smokers, if they were non-smokers at the time of stroke. Two patients (1 lacunar stroke and 1 cortical stroke) had missing data for smoking status. We fitted 4 further models: Model 1 (baseline + inflammation (INF)), Model 2 (baseline + endothelial dysfunction (END)), Model 3 (baseline + thrombosis/fibrinolysis (THR)) and Model 4 (baseline + INF + END + THR).

We compared each model for improvement over baseline. Model improvement was defined as a reduction in residual standard error (RSE) and increase in adjusted r-square (R^2).

We checked for multicollinearity between predictor variables using variance inflation factor. We checked model assumptions as follows: independence, linearity, constancy of variance and normality in the residuals. Alpha level for significance was $p < 0.05$. All analyses were performed with the statistical programming language R version 3.0.1 (http://www.r-project.org/) [27].

Meta-Analysis

We used the Review Manager 5 software (The Cochrane Collaboration) to update our prior meta-analysis [10], calculating the standardized mean difference (SMD) using the inverse variance method and a fixed effects model with 95% CIs.

Results

We recruited 125 patients: 65 with lacunar stroke and 60 with cortical stroke. The mean age of the total cohort was 66.4 ± 11.4 years and the median NIHSS score was 1 (Q1–Q3 1–2). The median time from stroke onset to blood sampling was 54.4 (Q1–Q3 36–74) days. Patient characteristics and plasma biomarkers by stroke subtype are listed in table 1.

Differences between Stroke Groups

The lacunar group had fewer men (39 vs. 51, p = 0.004), were younger (64 vs. 69 years, p = 0.015) and suffered less atrial fibrillation (2 vs. 9, p = 0.042) compared with the cortical group (table 1).

Plasma Biomarker Association with Lacunar Stroke

The lacunar group had lower t-PA levels compared with the cortical group (7.39 vs. 8.59 ng/ml, p = 0.029) in unadjusted analyses (table 1) and after adjustment for age, sex, hypertension, smoking, diabetes and atrial fibrillation (p = 0.035; table 2). There were no differences in the other plasma biomarkers between lacunar stroke and cortical stroke whether adjusted or not (online suppl. tables 2–4).

To determine if the reduced t-PA was related to smoking, we repeated the analysis in non-smokers only (lacunar stroke, n = 40 vs. cortical stroke, n = 46). t-PA levels remained lower in lacunar stroke (online suppl. table 5).

Table 1. Comparing patient characteristics and plasma biomarkers between lacunar and cortical stroke

	Lacunar stroke (n = 65)	Cortical stroke (n = 60)	p value
Male, n (%)	39 (60)	51 (85)	0.004*
Age, years, mean (SD)	64.1 (11.4)	69.0 (10.9)	0.015*
Hypertension, n (%)	37 (57)	39 (65)	0.458
Diabetes, n (%)	14 (21.5)	5 (8.3)	0.071
Current smoker, n (%)	24/64 (37.5)	13/59 (22.0)	0.095
NIHSS, median (Q1–Q3)	2 (1–3)	1 (0.75–2)	0.404
Time to sample, days, median (Q1–Q3)	56 (38–74)	52 (36–77)	0.894
Ischemic heart disease, n (%)	8 (12.3)	16 (26.6)	0.070
Atrial fibrillation, n (%)	2 (3.1)	9 (15)	0.042*
Hyperlipidemia, n (%)	26/64 (40.6)	23 (38.3)	0.939
Total cholesterol, mmol/l, mean (SD)	5.07 (1.10) (n = 57)	5.06 (1.13) (n = 53)	0.949
Positive family history of stroke, n (%)	10/64 (15.6)	4/58 (6.9)	0.220
Inflammation, median (Q1–Q3)			
CRP, mg/l	1.37 (0.84–3.44)	1.73 (0.97–3.54)	0.748
TNF, pg/ml	0.92 (0.72–1.33)	0.88 (0.76–1.23)	0.972
IL-6, pg/ml	2.57 (1.91–4.12) (n = 64)	2.58 (1.90–3.77)	0.994
Endothelial dysfunction, mean (SD)			
ICAM, ng/ml	162.78 (57.97)	159.27 (46.1) (n = 56)	0.711
vWF, IU/dl	129.31 (41.49)	131.7 (39.2)	0.741
Thrombosis/fibrinolysis			
Fibrinogen, g/l, mean (SD)	3.84 (0.61) (n = 64)	3.93 (0.67) (n = 59)	0.452
t-PA, ng/ml, mean (SD)	7.39 (3.13)	8.59 (2.92)	0.029*
D-dimer, ng/ml, median (Q1–Q3)	100 (73–157)	128.5 (73.25–182.5)	0.498

* $p < 0.05$.

Table 2. Association of t-PA with lacunar stroke subtype (n = 125)

	Regression coefficient (95% CI)	p value
Lacunar stroke subtype	−1.312 (−2.531 to −0.093)	0.035*
Age	−0.017 (−0.073 to 0.038)	0.530
Male sex	0.542 (−0.740 to 1.824)	0.404
Hypertension	0.393 (−0.806 to 1.593)	0.517
Smoking	1.027 (−0.277 to 2.333)	0.121
Diabetes	0.266 (−1.324 to 1.855)	0.741
Atrial fibrillation	0.240 (−1.854 to 2.334)	0.820

* $p < 0.05$.

Although the difference became non-significant when adjusted for age, sex, hypertension and diabetes, the change was slight, the regression coefficients and 95% CIs were similar [28] being −1.33 (95% CI −2.53 to −0.13) vs. −1.37 (95% CI −2.84 to 0.09) (table 2 and online suppl. table 6, respectively) and may reflect the reduced sample.

Meta-Analysis

On addition of our study to the 4 prior studies (new total 300 lacunar strokes), we show lower t-PA in lacunar versus non-lacunar stroke although the difference was not significant (fig. 1). Addition of our data moves the SMD from 0.02 (95% CI −0.18 to 0.21) to −0.08 (95% CI −0.25 to 0.09).

Biomarkers and WMH (All Patients: Lacunar and Cortical)

Quantitative WMH and plasma biomarkers were available for 98 patients. The baseline model (age, sex, hypertension and smoking status) explained 29% of the variance in quantitative WMH (RSE 1.081, R^2 0.289) with age, hypertension and smoking as significant predictors (table 3). Model 1 (baseline + INF) showed minor improvement over baseline (RSE 1.066, R^2 0.291). Models 2 (END), 3 (THR) and 4 (INF + END + THR) did not improve the baseline model. All models met model assumptions. There were no negative correlations between the components of the summed variables meaning a rise in one plasma marker was not offset by a fall in another (online suppl. table 7).

Table 3. Explaining variance in quantitative WMH with different predictor variables (n = 98)

Predictor variables	RSE	R^2
Baseline model		
Age‡, male sex, hypertension*, smoking†	1.081	0.289
Model 1		
Age‡, male sex, hypertension*, smoking† + inflammation	**1.066**	**0.291**
Model 2		
Age‡, male sex, hypertension*, smoking† + endothelial activation	1.098	0.285
Model 3		
Age‡, male sex, hypertension*, smoking† + thrombosis	1.098	0.278
Model 4		
Age‡, male sex, hypertension*, smoking† + inflammation + endothelial activation + thrombosis	1.094	0.285

Inflammation = log CRP, log TNF, log IL-6. Endothelial activation = vWF, ICAM. Thrombosis = t-PA, log D-dimer, fibrinogen. For RSE, bold indicates improvement over baseline, i.e. reduction in RSE. For R^2, bold indicates improvement over baseline, i.e. increase in R^2.

* $p < 0.05$, † $p < 0.01$, ‡ $p < 0.001$.

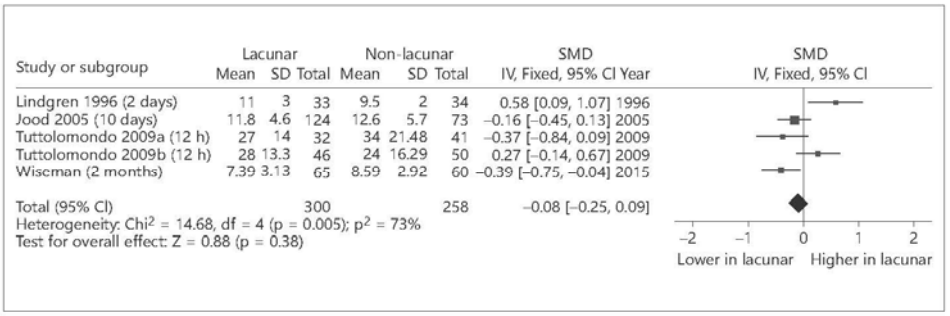


Fig. 1. Forest plot comparing t-PA levels between lacunar stroke and non-lacunar stroke. Values in bracket after study refers to time to blood draw.

Visually rated WMH correlated strongly with quantitative WMH (r = 0.84, 95% CI 0.77 to 0.89). Models for visually rated WMH show similar results to quantitative WMH (online suppl. table 8).

Discussion

We show a difference in t-PA levels between lacunar stroke and mild cortical stroke from plasma sampled well after the acute phase, independent of age, sex and risk factors. We did not find differences between stroke subtypes for biomarkers of inflammation (CRP, TNF and IL-6), endothelial dysfunction (vWF and ICAM) or other markers of hemostasis (fibrinogen and D-dimer). Except for a minor additional predictive effect of summed inflammatory markers, plasma biomarkers did not considerably improve the baseline model in explaining WMH.

t-PA

t-PA is a glycoprotein released mainly by endothelial cells [29, 30] to mediate the breakdown of thrombus. Its use as a thrombolytic agent might lead to the assumption that endogenous t-PA is protective against thrombosis [30]. However, higher t-PA antigen levels are associated

with the risk of coronary heart disease in generally healthy populations [29]. This may reflect increased endothelial disturbance resulting in increased t-PA secretion or else increased levels of its inhibitor, t-PA inhibitor (PAI-1), resulting in increased levels of circulating complexes with t-PA [29–31]. Although smoking did not influence outcome after recombinant t-PA in IST-3 [32] some [33, 34], but not all [35], observational studies suggest that smokers respond better to recombinant t-PA than non-smokers.

We found lower t-PA in those with lacunar as compared to those with cortical stroke. Reduced t-PA could mean lacunar stroke patients have reduced vascular damage – vWF levels were also lower in lacunar stroke but ICAM levels were higher (neither statistically significant). Alternatively, lacunar stroke patients might have increased endogenous fibrinolytic activity, if lower t-PA levels reflect lower levels of its inhibitor, PAI-1.

Knottnerus et al. [36] found significantly lower t-PA levels (and significantly higher PAI-1 levels) in 43 lacunar stroke patients with an isolated infarct versus 53 lacunar stroke patients with concurrent extensive WMH, hypothesizing that patients with extensive WMH lack the protective effect of PAI-1 in t-PA–induced tissue damage.

In our recent meta-analysis [10], t-PA was significantly higher in lacunar stroke patients than in non-stroke controls but did not differ significantly between patients of lacunar stroke and other stroke subtypes, although data are limited and the timing of sample collection could be confounding. Our samples were collected well after the acute phase and are more likely to reflect underlying pathway activity. The updated meta-analysis, including the current data, moves the evidence in favor of lower t-PA in lacunar stroke rather than non-lacunar stroke (fig. 1). The largest study to date to find lower levels of t-PA in small vessel stroke is the Sahlgrenska cohort, Sweden [37]: among 600 patients with ischemic stroke, including 124 with small vessel stroke, small vessel stroke patients had higher t-PA levels compared to non-stroke controls in the acute phase and at 3 months, but lower t-PA levels compared to patients with other stroke subtypes. The reduced t-PA is consistent with the impaired blood–brain barrier function found in the same cohort previously [38].

The lacunar group were significantly younger with fewer cases of atrial fibrillation and more smokers (non-significant) than the cortical group, although the association of lacunar stroke with lower t-PA was independent of these, and the pattern persisted in analysis restricted to non-smokers.

WMH and Biomarkers

Age, hypertension and smoking were significant predictors of WMH. The inflammatory biomarker summed variable appeared to improve the model (slight reduction in the RSE) but the additional explanatory power was small and could be interpreted as no model improvement. On the other hand, a similar effect size confirmed in a larger study would indicate a modest but important effect of plasma markers of inflammation on WMH prediction. The other plasma markers did not have any additional explanatory power. Studies that have measured WMH in non-stroke populations typically involve older people. We have clearly shown age to be the most important predictor variable in the assessment of WMH and thus correcting for age is crucial.

Two large studies [1, 12] showed independent associations between higher plasma inflammatory biomarkers and more WMH but had wide age ranges. However, Rouhl et al. [39] found no difference in CRP levels between 81 patients with and 265 patients without extensive WMH, Wersching et al. [13] found no association between CRP and WMH among 321 older stroke-free participants, Baune et al. [40] found no association between TNF and WMH among 268 community-dwelling participants and Aribisala et al. [41] found no association between inflammation (a latent factor comprising CRP, fibrinogen and IL-6) and WMH among 634 community-dwelling older people of near-identical age. Thus, it is possible that wide age ranges in some studies inflated associations between inflammatory markers and WMH. Shoamanesh et al. [15] found no association between some inflammatory biomarkers (including CRP, IL-6 and TNF) and SVD (defined as presence of silent infarcts and/or extensive WMH) in a large cohort of younger stroke-free Framingham participants (n = 522; mean age 60 years) but did associate ICAM with SVD. We found no association between ICAM and WMH in the present study but have much less power than the Framingham study. Our systematic review and meta-analysis [10] found no difference in ICAM levels between lacunar stroke and other stroke subtypes although only a few studies contributed data. ICAM was non-significantly higher in lacunar stroke patients than cortical stroke patients in the present study.

Conclusion

Despite being small (n = 125) and cross-sectional, our study uses thorough methods and adds new information. Our findings show a difference in t-PA levels between

lacunar and cortical stroke which should be verified in other data sets. Future studies should obtain plasma samples in the chronic phase after stroke and concentrate on longitudinal associations, especially the role of t-PA in stroke subtypes as it could help explain mechanisms. A large prospective study of accurately phenotyped stroke patients would be helpful. It is important to not only control for age specifically, but also hypertension and smoking when modeling features of SVD such as WMH and biomarkers.

Funding

This study was supported by the Chief Scientist Office of the Scottish Government (CZB/4/281) (J.M.W. and M.S.D.), the Wellcome Trust (075611) (F.N.D. and J.M.W.) and a Principal's Career Development PhD Scholarship from the University of Edinburgh (S.J.W.).

Disclosure Statement

The authors have no conflicts of interest.

References

1 Satizabal CL, Zhu YC, Mazoyer B, Dufouil C, Tzourio C: Circulating IL-6 and CRP are associated with MRI findings in the elderly: the 3C-Dijon study. Neurology 2012;78:720–727.
2 Knottnerus IL, Ten Cate H, Lodder J, Kessels F, van Oostenbrugge RJ: Endothelial dysfunction in lacunar stroke: a systematic review. Cerebrovasc Dis 2009;27:519–526.
3 Wardlaw JM, Smith C, Dichgans M: Mechanisms of sporadic cerebral small vessel disease: insights from neuroimaging. Lancet Neurol 2013;12:483–497.
4 Markus HS, Hunt B, Palmer K, Enzinger C, Schmidt H, Schmidt R: Markers of endothelial and hemostatic activation and progression of cerebral white matter hyperintensities: longitudinal results of the Austrian Stroke Prevention study. Stroke 2005;36:1410–1414.
5 Stevenson SF, Doubal FN, Shuler K, Wardlaw JM: A systematic review of dynamic cerebral and peripheral endothelial function in lacunar stroke versus controls. Stroke 2010;41:e434–e442.
6 Debette S, Markus HS: The clinical importance of white matter hyperintensities on brain magnetic resonance imaging: systematic review and meta-analysis. BMJ 2010;341:c3666.
7 Wardlaw JM, Doubal FN, Valdes-Hernandez M, Wang X, Chappell FM, Shuler K, et al: Blood-brain barrier permeability and long-term clinical and imaging outcomes in cerebral small vessel disease. Stroke 2013;44:525–527.
8 Whiteley W, Chong WL, Sengupta A, Sandercock P: Blood markers for the prognosis of ischemic stroke: a systematic review. Stroke 2009;40:e380–e389.
9 Castellanos M, Serena J: Applicability of biomarkers in ischemic stroke. Cerebrovasc Dis 2007;24(suppl 1):7–15.
10 Wiseman S, Marlborough F, Doubal F, Webb DJ, Wardlaw J: Blood markers of coagulation, fibrinolysis, endothelial dysfunction and inflammation in lacunar stroke versus non-lacunar stroke and non-stroke: systematic review and meta-analysis. Cerebrovasc Dis 2014;37:64–75.
11 Hasan N, McColgan P, Bentley P, Edwards RJ, Sharma P: Towards the identification of blood biomarkers for acute stroke in humans: a comprehensive systematic review. Br J Clin Pharmacol 2012;74:230–240.
12 van Dijk EJ, Prins ND, Vermeer SE, Vrooman HA, Hofman A, Koudstaal PJ, et al: C-reactive protein and cerebral small-vessel disease: the Rotterdam Scan study. Circulation 2005;112:900–905.
13 Wersching H, Duning T, Lohmann H, Mohammadi S, Stehling C, Fobker M, et al: Serum C-reactive protein is linked to cerebral microstructural integrity and cognitive function. Neurology 2010;74:1022–1029.
14 Schmidt R, Schmidt H, Pichler M, Enzinger C, Petrovic K, Niederkorn K, et al: C-reactive protein, carotid atherosclerosis, and cerebral small-vessel disease: results of the Austrian Stroke Prevention study. Stroke 2006;37:2910–2916.
15 Shoamanesh A, Preis SR, Beiser AS, Vasan RS, Benjamin EJ, Kase CS, et al: Inflammatory biomarkers, cerebral microbleeds, and small vessel disease: Framingham Heart study. Neurology 2015;84:825–832.
16 Nagai M, Hoshide S, Kario K: Association of prothrombotic status with markers of cerebral small vessel disease in elderly hypertensive patients. Am J Hypertens 2012;25:1088–1094.
17 Kim JS, Lee HS, Park HY, Kim SS, Kang HG, Kim NH, et al: Endothelial function in lacunar infarction: a comparison of lacunar infarction, cerebral atherosclerosis and control group. Cerebrovasc Dis 2009;28:166–170.
18 Wardlaw JM, Smith EE, Biessels GJ, Cordonnier C, Fazekas F, Frayne R, et al: Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration. Lancet Neurol 2013;12:822–838.
19 Doubal FN, Dhillon B, Dennis MS, Wardlaw JM: Retinopathy in ischemic stroke subtypes. Stroke 2009;40:389–393.
20 Brott T, Adams HP Jr, Olinger CP, Marler JR, Barsan WG, Biller J, et al: Measurements of acute cerebral infarction: a clinical examination scale. Stroke 1989;20:864–870.
21 Bamford J, Sandercock P, Dennis M, Burn J, Warlow C: Classification and natural history of clinically identifiable subtypes of cerebral infarction. Lancet 1991;337:1521–1526.
22 Mead GE, Lewis SC, Wardlaw JM, Dennis MS, Warlow CP: How well does the Oxfordshire community stroke project classification predict the site and size of the infarct on brain imaging? J Neurol Neurosurg Psychiatry 2000;68:558–562.
23 Fazekas F, Chawluk JB, Alavi A, Hurtig HI, Zimmerman RA: MR Signal abnormalities at 1.5 T in Alzheimer's dementia and normal aging. AJR Am J Roentgenol 1987;149:351–356.
24 Potter G, Morris Z, Wardlaw J: Enlarged Perivascular Spaces (EPVS): a visual rating scale and user guide. http://www.bric.ed.ac.uk/documents/epvs-rating-scale-user-guide.pdf (accessed March 23, 2014).
25 Cordonnier C, Potter GM, Jackson CA, Doubal F, Keir S, Sudlow CL, et al: Improving interrater agreement about brain microbleeds: development of the Brain Observer MicroBleed Scale (BOMBS). Stroke 2009;40:94–99.
26 Hernández Mdel C, Ferguson KJ, Chappell FM, Wardlaw JM: New multispectral MRI data fusion technique for white matter lesion segmentation: method and comparison with thresholding in FLAIR images. Eur Radiol 2010;20:1684–1691.
27 R Core Team: R: A Language and Environment for Statistical Computing. Vienna, Austria, R Foundation for Statistical Computing, 2013.
28 Gardner MJ, Altman DG: Confidence intervals rather than P values: estimation rather than hypothesis testing. Br Med J (Clin Res Ed) 1986;292:746–750.
29 Willeit P, Thompson A, Aspelund T, Rumley A, Eiriksdottir G, Lowe G, et al: Hemostatic factors and risk of coronary heart disease in general populations: new prospective study and updated meta-analyses. PLoS One 2013;8:e55175.
30 de Bono D: Significance of raised plasma concentrations of tissue-type plasminogen activator and plasminogen activator inhibitor in patients at risk from ischaemic heart disease. Br Heart J 1994;71:504–507.

31 Chandler WL, Jascur ML, Henderson PJ: Measurement of different forms of tissue plasminogen activator in plasma. Clin Chem 2000;46:38–46.
32 The IST-3 Collaborative Group, Sandercock P, Wardlaw JM, et al: The benefits and harms of intravenous thrombolysis with recombinant tissue plasminogen activator within 6 h of acute ischaemic stroke (the third international stroke trial [IST-3]): a randomised controlled trial. Lancet 2012; 379:2352–2363.
33 Kvistad CE, Oeygarden H, Logallo N, Thomassen L, Waje-Andreassen U, Naess H: Is smoking associated with favourable outcome in tPA-treated stroke patients? Acta Neurol Scand 2014;130:299–304.
34 Kufner A, Nolte CH, Galinovic I, Brunecker P, Kufner GM, Endres M, et al: Smoking-thrombolysis paradox: recanalization and reperfusion rates after intravenous tissue plasminogen activator in smokers with ischemic stroke. Stroke 2013;44:407–413.
35 Aries MJ, Uyttenboogaart M, Koch MW, Langedijk M, Vroomen PC, Luijckx GJ, et al: Does smoking influence outcome after intravenous thrombolysis for acute ischaemic stroke? Eur J Neurol 2009;16:819–822.
36 Knottnerus IL, Govers-Riemslag JW, Hamulyak K, Rouhl RP, Staals J, Spronk HM, et al: Endothelial activation in lacunar stroke subtypes. Stroke 2010;41:1617–1622.
37 Jood K, Ladenvall P, Tjärnlund-Wolf A, Ladenvall C, Andersson M, Nilsson S, et al: Fibrinolytic gene polymorphism and ischemic stroke. Stroke 2005;36:2077–2081.
38 Wardlaw JM, Doubal F, Armitage P, Chappell F, Carpenter T, Muñoz Maniega S, et al: Lacunar stroke is associated with diffuse blood-brain barrier dysfunction. Ann Neurol 2009; 65:194–202.
39 Rouhl RP, Damoiseaux JG, Lodder J, Theunissen RO, Knottnerus IL, Staals J, et al: Vascular inflammation in cerebral small vessel disease. Neurobiol Aging 2012;33:1800–1806.
40 Baune BT, Ponath G, Rothermundt M, Roesler A, Berger K: Association between cytokines and cerebral MRI changes in the aging brain. J Geriatr Psychiatry Neurol 2009;22: 23–34.
41 Aribisala BS, Wiseman S, Morris Z, Valdés-Hernández MC, Royle NA, Maniega SM, et al: Circulating inflammatory markers are associated with magnetic resonance imaging-visible perivascular spaces but not directly with white matter hyperintensities. Stroke 2014; 45:605–607.

"Cerebrovascular disease in rheumatic diseases: A systematic review and meta-analysis" (see Chapter 4). Reproduced with permission from the publisher Wolters Kluwer.

Original Contribution

Cerebrovascular Disease in Rheumatic Diseases

A Systematic Review and Meta-Analysis

Stewart J. Wiseman, BSc, Stuart H. Ralston, FRCP, Joanna M. Wardlaw, FRCR

Background and Purpose—Some rheumatic diseases are associated with stroke. Less is known about associations with stroke subtypes or stroke risk by age. We quantified the association between stroke, its subtypes, and rheumatic diseases and identified when stroke risk is greatest.

Methods—Searches of EMBASE (from 1980) and MEDLINE (from inception) to end 2014 and manual search of reference lists for studies of stroke and stroke subtypes in rheumatic diseases as well as studies measuring cerebrovascular disease from magnetic resonance imaging.

Results—Prior published meta-analyses and new pooled analyses of any stroke in rheumatoid arthritis, systemic lupus erythematosus, ankylosing spondylitis, gout, and psoriasis show an excess risk of stroke over the general population with odds ratio (OR) ranging from 1.51 (95% confidence interval: 1.39–1.62) to 2.13 (1.53–2.98). New meta-analyses of stroke subtypes in rheumatoid arthritis [ischemic: OR, 1.64 (1.32–2.05); hemorrhagic: OR, 1.68 (1.11–2.53)] and systemic lupus erythematosus [ischemic: OR, 2.11 (1.66–2.67); hemorrhagic: OR, 1.82 (1.07–3.09)] show an excess risk of stroke over the general population. Stroke risk across rheumatic diseases is highest in those aged <50 years [OR, 1.79 (1.46–2.20)] and reduces relatively with ageing [>65 years: OR, 1.14 (0.94–1.38); difference $P<0.007$]. Inflammatory arthropathies conveyed higher stroke risk than noninflammatory diseases (OR, 1.3, 1.2–1.3). It was not possible to adjust ORs for risk factors or treatments.

Conclusions—Risk of any stroke is higher in most rheumatic diseases than in the general population, particularly <50 years. Rheumatoid arthritis and systemic lupus erythematosus increase ischemic and hemorrhagic stroke risk by 60% to 100% relative to the general population. **(*Stroke*. 2016;47:00-00. DOI: 10.1161/STROKEAHA.115.012052.)**

Key Words: arthritis ■ atrophy ■ inflammation ■ rheumatology ■ stroke

Stroke is a major health problem. Overall incidence rates are falling,[1,2] but better access to medical care and improvements in secondary prevention increase survival, so stroke prevalence, and thus health-care costs, remain high. An ageing population will increase this trend.

Rheumatic diseases such as rheumatoid arthritis (RA) are an independent risk factor for stroke.[3,4] People with these diseases die prematurely from cardiovascular disease including stroke,[5,6] so an understanding of stroke risk among these patients is needed to reduce mortality. However, data linking rheumatic diseases with higher stroke risk are based mostly on stroke reported from large population studies, ie, a composite outcome of any stroke. Less is known about associations between rheumatic diseases (inflammatory or noninflammatory) and major stroke subtypes whose mechanisms differ, eg, ischemic versus hemorrhagic stroke, or large artery atheromatous versus intrinsic small vessel ischemic stroke, or with conditions associated with cerebral small vessel disease (SVD), such as cognitive decline and gait disturbances.[7,8]

Population stroke incidence rises with age. Stroke early in life is rare, yet most of the stroke associated with rheumatic diseases seems to be at younger ages[9–15] and may level off, as some studies[12,13,16,17] report no risk difference in those >65 years. However, there is currently no meta-analysis on the overall association of rheumatic diseases with stroke by age. Clarifying timing of greatest stroke risk has important clinical implications.

Studies to date do not fully explain the increased stroke risk among rheumatic populations by vascular risk factors.[18,19] A proportion of stroke risk in rheumatic diseases could relate to the higher inflammatory activity seen in many arthropathies, which is systemic, nonresolving, and often only controlled with aggressive antirheumatic drugs. Inflammation, therefore, plausibly explains some of the excess risk because of atheromatous stroke as inflammation is involved in all stages of atherosclerosis from fatty streak formation to plaque disruption.[20–22] The role of inflammation in SVD is less certain, but inflammation is seen pathologically in the perforating

Received November 4, 2015; final revision received February 2, 2016; accepted February 3, 2016.

From the Centre for Clinical Brain Sciences, University of Edinburgh (S.J.W., J.M.W.), Centre for Genomic and Experimental Medicine (S.H.R.), University of Edinburgh, Edinburgh, United Kingdom.

The online-only Data Supplement is available with this article at http://stroke.ahajournals.org/lookup/suppl/doi:10.1161/STROKEAHA.115.012052/-/DC1.

Correspondence to Joanna M. Wardlaw, FRCR, Centre for Clinical Brain Sciences, Chancellor's Building, Royal Infirmary of Edinburgh, 49 Little France Crescent, Edinburgh EH16 4SB, United Kingdom. E-mail Joanna.Wardlaw@ed.ac.uk

***Stroke* is available at http://stroke.ahajournals.org** **DOI: 10.1161/STROKEAHA.115.012052**

arteriolar walls and perivascular tissue.[23,24] Endothelial damage is a primary step in atherosclerosis and SVD and factors that contribute to endothelial damage (eg, immune complex formation and complement activation) are also seen in rheumatic diseases.

Our aims are to review associations between stroke and rheumatic disease, to summarize incidence rates for stroke subtypes, to calculate pooled rate ratios for stroke subtypes versus the general population, to see if risk is greatest at specific ages; and to determine if rheumatic diseases increase the risk of silent vascular disease on neuroimaging.

Methods

Study Design

We used a systematic approach to assess stroke and stroke subtypes as the outcome measure and various rheumatic diseases as the exposure. Research ethics committee approval was not required. The study was not registered in any database.

Data Sources

We prepared this review in accordance with the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement.[25] We used structured MeSH search terms (Table I in the online-only Data Supplement) in Ovid to search EMBASE (from 1980) and MEDLINE (from inception) to 2014 on 14 December 2014. Data were extracted in accordance with MOOSE (Meta-Analyses and Systematic Reviews of Observational Studies) guidelines.[26] We categorized magnetic resonance imaging (MRI) findings according to STRIVE (Standards for Reporting Vascular Changes on Neuroimaging) guidelines.[27] We identified additional papers from reference lists and contacted authors for additional data when required.

Study Selection

We included English language studies reporting on stroke in rheumatic disease and studies that assessed brain imaging features of SVD on MRI. We excluded studies using functional MRI, positron emission tomography, single photon emission computed tomography, and Doppler ultrasound.

Data Extraction

We extracted data on study population demographics, control groups, stroke type (ischemic/hemorrhagic), ischemic stroke subtypes (large artery/lacunar), findings on stroke risk relative to a comparator group, and MRI findings. We noted if studies controlled for age and vascular risk factors.

Quality Assessment

We developed a checklist adapted from STROBE (Strengthening the Reporting of Observational Studies in Epidemiology)[28] to assess the quality of included studies (Table II in the online-only Data Supplement).

Data Synthesis and Analysis

We defined SVD from MRI features as per recent STRIVE neuroimaging standards,[27] being any of recent small subcortical infarcts, white matter hyperintensities (WMH), lacunes, microbleeds, prominent peri-vascular spaces, or atrophy. Clinically, patients might show no symptoms, or they might suffer cognitive impairment or other neurological involvement in addition to stroke (lacunar stroke accounts for ≈25% of all ischemic strokes[29]).

We defined stroke incidence rates as number of strokes as a function of a follow-up period and stroke rate ratios as the ratio of stroke incidence rate in the observed group (eg, RA) over incidence rates in the general population. We used unadjusted (crude) rates throughout as different studies controlled for different variables making comparison of uniform adjustments impossible.

We recorded stroke incidence rates when reported and calculated them when not reported but where data (ie, number of stroke events and a follow-up period) were available. We converted all incidence rates to per 100 000 person-years. If follow-up duration (in patient-years) was not specifically reported, we estimated this by multiplying number of patients by the average years of follow-up.

Where a rheumatic disease had contributing data from >1 study, we pooled incidence rates by taking the range of available values, which did not allow us to assess heterogeneity. Next, we calculated a point estimate for incidence rate per 100 000 person-years for individual rheumatic diseases based on the weighted mean, using study size as the weighting factor and then estimated a 95% confidence interval (CI) based on the Poisson distribution as implemented in the epitools package for the statistical programming language R version 3.0.1 (http://www.r-project.org/).[30]

We calculated rate ratios using the Cochrane Collaboration's Review Manager 5 software when not reported but where required data were available.

Some studies did not provided number of strokes and number of patient-years observed for the control group but instead only provided the rate ratio together with a CI. As per Cochrane Handbook,[31] CI can be converted to standard errors and the natural logarithms of rate ratios may be combined across studies using the generic inverse-variance method. We used this approach to pool stroke risk for ischemic stroke and hemorrhagic stroke and for the age category pooled analysis.

We assessed between-study heterogeneity using the I^2 statistic. We used random effects models in all meta-analyses.

Results

The search returned 434 titles and abstracts; 69 papers were reviewed in full and 23 studies contributed data to new meta-analyses. We excluded studies that did not measure stroke with appropriate imaging (n=12), pathology studies (n=5), guidelines and review papers (n=4), and small studies (<50 patients) that only described imaging features of SVD (n=25) (Figure I in the online-only Data Supplement).

Any Stroke

Prior meta-analyses and large registry studies of any stroke are reported for completeness (Table III in the online-only Data Supplement). RA [incidence rate ratio (RR), 1.91; 95% CI, 1.73–2.12], ankylosing spondylitis [odds ratio (OR), 1.51; 1.39–1.62] and gout (RR, 1.71; 1.68–1.75), but not osteoarthritis (OA; OR, 1.11; 0.95–1.29), showed higher risk of stroke than the general population.

We add new meta-analyses on any stroke in systemic lupus erythematosus (SLE), psoriasis, and psoriatic arthritis. The pooled odds of any stroke in SLE from 5 studies[13,17,32–34] (772 strokes, 40 652 SLE patients) was 2.13 (1.53–2.98) (Figure II in the online-only Data Supplement). We updated 2 psoriasis meta-analyses[35,36] and added a new meta-analysis for psoriatic arthritis. The pooled odds of any stroke in psoriasis (9 studies[37–45]; 6925 strokes; 400 767 patients) was 1.08 (1.00–1.16) (Figure III in the online-only Data Supplement), and of any stroke in psoriatic arthritis (3 studies[42,43,46] 217 strokes; 12 051 patients) was 1.27 (0.98–1.64) (Figure III in the online-only Data Supplement).

RA (prototypical inflammatory rheumatic disease) showed significant risk of stroke over the general population (n=26 143

patients; OR, 1.91; 1.73–2.12)[47] whereas OA (degenerative) did not (n=40 817 patients; OR, 1.11; 0.95–1.29).[48] In direct comparison, patients with OA alone had lower stroke risk (11 633 RA versus 163 274 OA patients; OR, 1.3; 1.2–1.3, ie, higher stroke risk in RA).[49]

Stroke Subtypes: Ischemic and Hemorrhagic

Incidence

Table summarizes our meta-analysis of incidence of ischaemic[13–15,17,33,50–57] and haemorrhagic[13–15,17,33,52,53] stroke by different rheumatic diseases with a general population comparator.[58]

Stroke Risk: Pooled Rate Ratios

Sufficient data to perform meta-analysis of rate ratios for stroke incidence among stroke subtypes versus the general population were only available for RA and SLE. In RA versus the general population, the pooled odds of ischemic stroke (3481 strokes; 86 280 patients) and hemorrhagic stroke (562 strokes; 84 419 patients) were 1.64 (95% CI, 1.32–2.05) and 1.68 (1.11–2.53), respectively (Figures 1 and 2). In SLE versus the general population, the pooled odds of ischemic stroke (945 strokes; 55 699 patients) and hemorrhagic stroke (164 strokes; 44 062 patients) were 2.11 (1.66–2.67) and 1.82 (1.07–3.09), respectively (Figures 1 and 2). F1,F2

Subtypes of Ischemic Stroke

For SLE, 2 studies[17,50] provide incidence rate data for *ischemic* stroke subtypes: among 490 SLE patients,[17] 13 had cortical strokes (equivalent to 271 cortical strokes per 100 000 person-years) and 4 had lacunar strokes (83 per 100,000) from 4802 person-years follow-up; among 232 SLE patients,[50] 20 had large artery strokes (1150 per 100 000), 17 had small vessel

Table. Stroke Incidence Rates by Stroke Subtype Among Different Rheumatic Diseases

Rheumatic Disease	Included Studies	Strokes	Person-Years Follow-Up	Range of Incident Rates, per 100 000 Person-Years	Mean IR, per 100 000 Person-Years (95% CI)	References
Ischemic						
Rheumatoid arthritis	6	3611	1 193 249	178–1077	303 (269–337)	14,51–55
Gout	1	5391	767 725	702	702 (650–754)	15
Ankylosing spondylitis	1	111	76 494	145	145 (121–169)	52
Reiter's	1	13	7436	175	175 (149–201)	52
Psoriasis/PsA	None					
Polyarteritis nodosa	1	46	18 106	254	254 (223–285)	52
Ploymyalgia	1	1777	362 912	489	489 (446–532)	52
SLE	7	995	271 076	208–2530	367 (329–404)	13,17,33,50,52,56,57
Scleroderma	1	44	11 264	391	391 (352–430)	52
Sjogren's	1	68	28 600	238	238 (208–268)	52
Osteoarthritis	None					
General population					141 (127–156)	58
Hemorrhagic						
Rheumatoid arthritis	3	562	1 096 594	43–118	51 (37–65)	14,52,53
Gout	1	1864	767 725	242	242 (211–272)	15
Ankylosing spondylitis	1	42	76 494	55	55 (40–69)	52
Reiter's	1	2	7436	27	27 (17–37)	52
Psoriasis/PsA	None					
Polyarteritis nodosa	1	5	18 106	28	28 (18–38)	52
Polymyalgia	1	204	362 912	56	56 (41–71)	52
SLE	4	164	223 027	35–118	74 (57–91)	13,17,33,52
Scleroderma	1	8	11 264	71	71 (54–87)	52
Sjogren's	1	5	28 600	17	17 (9–25)	52
Osteoarthritis	None					
General population					12 (9–17)	58

CI indicates confidence interval; Mean IR, mean incident rate (weight based on study size, ie, person-years observed); PsA, psoriatic arthritis; SLE, systemic lupus erythematosus.

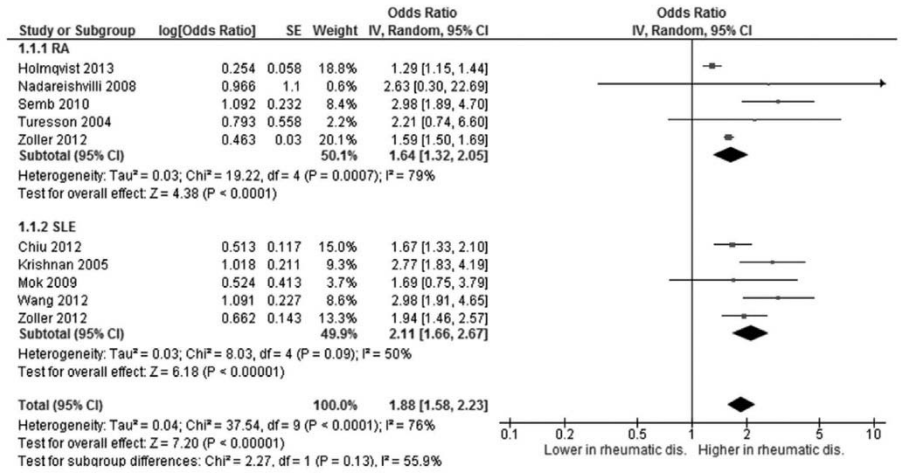


Figure 1. Forest plot—ischemic stroke in rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) versus general population.[13,14,17,33,52–55,57] CI indicates confidence interval.

strokes (997 per 100 000) and 4 had cardioembolic strokes (230 per 100 000) from 1739 person-years follow-up. There are limited comparative data from the general population but Sacco et al[59] report annual incidence rates for lacunar stroke as 33 per 100 000 population. There were insufficient data to pool rate ratios among ischemic stroke subtypes.

Age, Rheumatic Disease and Stroke

The pooled odds of any stroke (11 879 strokes; 340 548 patients)[9,12–17,33,60] across 5 rheumatic diseases (data were available for RA, SLE, psoriasis, ankylosing spondylitis, and gout) versus the general population was 1.38 (95% CI, 1.21–1.57) (Figure 3). When split by age, the pooled odds were 1.79 (1.46–2.20) for age <50 years, 1.49 (1.07–2.06) for age 50 to 65 and 1.14 (0.94–1.38) for age 65 and above (Figure 3). The age categories were significantly different (χ^2 test for subgroups, P=0.007). We include a study by study review of stroke by age categories across stroke in general and by ischemic and hemorrhagic stroke subtypes in Table IV in the online-only Data Supplement.

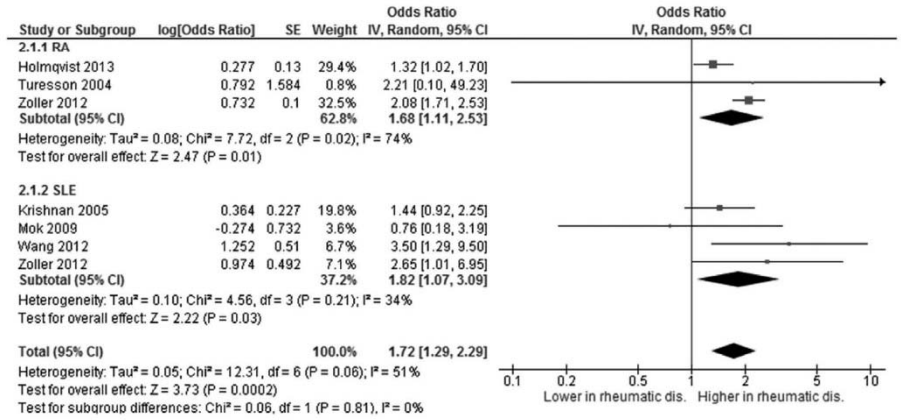


Figure 2. Forest plot—hemorrhagic stroke in rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) versus general population.[13,14,17,33,52,53] CI indicates confidence interval.

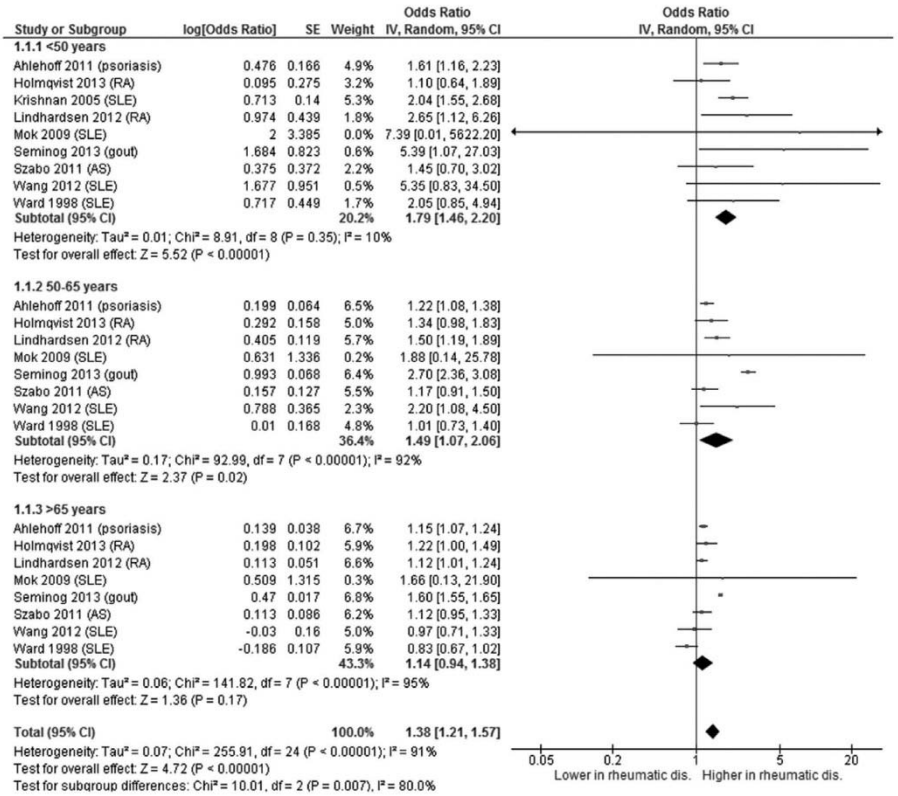


Figure 3. Forest plot—any stroke in rheumatic diseases by 3 age categories.[9,12–17,33,60] AS indicates ankylosing spondylitis; CI, confidence interval; RA, rheumatoid arthritis; and SLE, systemic lupus erythematosus.

Structural MRI brain imaging findings

We reviewed numerous MRI studies of the brain in rheumatic diseases. The details are provided in Table V in the online-only Data Supplement. Most were small, many investigated neurologically symptomatic patients only (for example comparing neurolupus with SLE), and few controlled for age and known vascular risk factors.

Discussion

Brain damage from any type of stroke, from ischemic and hemorrhagic stroke, and silent vascular damage such as WMIIs is increased in most rheumatic diseases. Most data are for stroke in general, but ischemic and hemorrhagic strokes were also increased in our new pooled analyses of RA and SLE, as were silent vascular disease markers.

RA, SLE, AS, gout, and to a lesser degree psoriasis carry a higher risk of stroke over the general population. Stroke incidence varies across rheumatic diseases (Table) and seem higher than the general population (eg, Rothwell et al[58] report annual incidence rates for ischemic and hemorrhagic stroke as 141 (95% CI, 127–156) and 12 (9–17) per 100 000 population, respectively).

Rheumatic disease patients aged <50 have a particularly high stroke risk compared with the general population. There was no additional stroke risk in OA. Other rheumatic diseases are understudied. Although increased stroke risk may reflect impact on lifestyle through the physical effects of rheumatic diseases, the possibility that increased systemic inflammation affects the brain directly is suggested by the higher stroke risk in inflammatory versus noninflammatory arthropathies. A better understanding of stroke in rheumatic disease would help focus clinical practice on prevention of vascular brain damage, including early lifestyle interventions and any vascular prevention role for anti-inflammatory agents, in these patients.

This is the first analysis to quantify stroke subtype rates and risk in rheumatic disease including by age. Our results are

comparable with Holmqvist et al's[61] meta-analysis of stroke in SLE, including that stroke risk is increased in the patients aged <50, but we include other rheumatic diseases. We were limited by the different methods used in the primary studies although we attempted to correct for this by using random effects meta-analysis. Consistency of reporting of stroke rates is a recognised problem and future studies should attempt to standardize their methods.[62]

The increase in risk of vascular disease in RA and SLE is perhaps expected, but we also note the almost doubling of stroke in gout (1.71; 1.68–1.75; n=9951 strokes, n=202 033 patients) over the general population perhaps because of the relationship between gout and metabolic syndrome, or to uric acid's independent association with ischemic and hemorrhagic stroke subtypes.[63]

The review's strengths include data mostly from large population-based studies although we only included English language publications. We could not adjust for vascular risk factors or treatments, limiting generalizability, and cannot exclude the possibility of study bias. Patients with rheumatic disease are often assiduously monitored (because of the disease and treatments), and so might have minor neurological problems investigated more compared with the general population.

Data on ischemic stroke subtypes were limited to 2 studies in SLE.[17,50] Many more studies reported on SVD features among patients with several rheumatic diseases. Although small size and disparate reporting precluded meta-analysis, the general impression was of more vascular lesions in rheumatic diseases.

The increased stroke risk at earlier ages uses data from 340 548 patients (11 879 strokes). The excess risk was almost double that of the general population, highest in those <50 years, and declined steadily to approach that of the general population >65. However, there was heterogeneity in study reporting and inconsistencies in age categories (despite guidance to use mid-decade age bands[62]). The clear trend for higher stroke risk <50 years suggests that atherosclerosis is unlikely to be the sole pathogenic driver. Systemic inflammation may play a role. The increased risk at younger ages might reflect more rheumatic disease activity before the inflammation is well controlled.

The risk of any stroke, ischemic or hemorrhagic stroke, and MRI findings seem worse in inflammatory arthropathies (RA, SLE, AS, gout, psoriatic arthritis) than noninflammatory arthropathies (OA). Inflammation is a risk factor for stroke[64] and plasma markers of inflammation (C-reactive protein, tumor necrosis factor-a, interleukin-6) are associated with stroke[65] and increased WMH burden. Antiphospholipid antibodies increase the risk of thrombus: in RA, strokes (and preclinical brain abnormalities such as WMH) are more common in those with antiphospholipid than those without.[66–68] Therefore, inflammation generated in the rheumatic diseases may be at least in part responsible for the marked stroke risk and overt brain lesions, and increased risk at younger ages.

A large brain imaging population study with detailed stroke phenotyping is needed to fully characterize stroke subtypes including SVD in rheumatic patients. A clear picture of increased stroke risk in younger patients is established indicating a need to a) unravel the extent to which inflammation, lifestyle, antirheumatic treatments, and risk factors (traditional as well as new) contribute to stroke risk and b) whether aggressive management of these risk factors including inflammation can ameliorate the stroke risk. Imaging features such as WMH might assist in identifying rheumatic disease patients who are at particularly high risk of stroke, as do WMH in the general ageing population.[69]

Sources of Funding

This work was supported by the Brain Research Imaging Centre, University of Edinburgh. The first author is also supported by a University of Edinburgh Principal's Career Development Scholarship. All authors designed the study and agreed on the final manuscript for publication.

Disclosures

None.

References

1. NHS National Services Scotland—Information Services Division. Stroke. Statistics Update; 2015:1–43.
2. Koton S, Schneider AL, Rosamond WD, Shahar E, Sang Y, Gottesman RF, et al. Stroke incidence and mortality trends in US communities, 1987 to 2011. *JAMA*. 2014;312:259–268. doi: 10.1001/jama.2014.7692.
3. JBS3 Board. Joint British Societies' consensus recommendations for the prevention of cardiovascular disease (JBS3). *Heart*. 2014;100:ii1–ii67.
4. Peters MJ, Symmons DP, McCarey D, Dijkmans BA, Nicola P, Kvien TK, et al. EULAR evidence-based recommendations for cardiovascular risk management in patients with rheumatoid arthritis and other forms of inflammatory arthritis. *Ann Rheum Dis*. 2010;69:325–331. doi: 10.1136/ard.2009.113696.
5. Gabriel SE. Why do people with rheumatoid arthritis still die prematurely? *Ann Rheum Dis*. 2008;67 Suppl 3:iii30–iii34. doi: 10.1136/ard.2008.098038.
6. Yurkovich M, Vostretsova K, Chen W, Aviña-Zubieta JA. Overall and cause-specific mortality in patients with systemic lupus erythematosus: a meta-analysis of observational studies. *Arthritis Care Res (Hoboken)*. 2014;66:608–616. doi: 10.1002/acr.22173.
7. Wardlaw JM, Smith C, Dichgans M. Mechanisms of sporadic cerebral small vessel disease: insights from neuroimaging. *Lancet Neurol*. 2013;12:483–497. doi: 10.1016/S1474-4422(13)70060-7.
8. Pantoni L. Cerebral small vessel disease: from pathogenesis and clinical characteristics to therapeutic challenges. *Lancet Neurol*. 2010;9:689–701. doi: 10.1016/S1474-4422(10)70104-6.
9. Lindhardsen J, Ahlehoff O, Gislason GH, Madsen OR, Olesen JB, Svendsen JH, et al. Risk of atrial fibrillation and stroke in rheumatoid arthritis: Danish nationwide cohort study. *BMJ*. 2012;344:e1257.
10. Solomon DH, Goodson NJ, Katz JN, Weinblatt ME, Avorn J, Setoguchi S, et al. Patterns of cardiovascular risk in rheumatoid arthritis. *Ann Rheum Dis*. 2006;65:1608–1612. doi: 10.1136/ard.2005.050377.
11. Bessant R, Hingorani A, Patel L, MacGregor A, Isenberg DA, Rahman A. Risk of coronary heart disease and stroke in a large British cohort of patients with systemic lupus erythematosus. *Rheumatology (Oxford)*. 2004;43:924–929. doi: 10.1093/rheumatology/keh213.
12. Ward MM. Premature morbidity from cardiovascular and cerebrovascular diseases in women with systemic lupus erythematosus. *Arthritis Rheum*. 1999;42:338–346.
13. Wang IK, Muo CH, Chang YC, Liang CC, Lin SY, Chang CT, et al. Risks, subtypes, and hospitalization costs of stroke among patients with systemic lupus erythematosus: a retrospective cohort study in Taiwan. *J Rheumatol*. 2012;39:1611–1618. doi: 10.3899/jrheum.111510.
14. Holmqvist M, Gränsmark E, Mantel A, Alfredsson L, Jacobsson LT, Wallberg-Jonsson S, et al. Occurrence and relative risk of stroke in incident and prevalent contemporary rheumatoid arthritis. *Ann Rheum Dis*. 2013;72:541–546. doi: 10.1136/annrheumdis-2012-201387.
15. Seminog OO, Goldacre MJ. Gout as a risk factor for myocardial infarction and stroke in England: evidence from record linkage studies. *Rheumatology (Oxford)*. 2013;52:2251–2259. doi: 10.1093/rheumatology/kct293.

16. Szabo SM, Levy AR, Rao SR, Kirbach SE, Lacaille D, Cifaldi M, et al. Increased risk of cardiovascular and cerebrovascular diseases in individuals with ankylosing spondylitis: a population-based study. *Arthritis Rheum*. 2011;63:3294–3304. doi: 10.1002/art.30581.
17. Mok CC, Ho LY, To CH. Annual incidence and standardized incidence ratio of cerebrovascular accidents in patients with systemic lupus erythematosus. *Scand J Rheumatol*. 2009;38:362–368. doi: 10.1080/03009740902776927.
18. Turesson C, Jacobsson LT, Matteson EL. Cardiovascular co-morbidity in rheumatic diseases. *Vasc Health Risk Manag*. 2008;4:605–614.
19. Esdaile JM, Abrahamowicz M, Grodzicky T, Li Y, Panaritis C, du Berger R, et al. Traditional Framingham risk factors fail to fully account for accelerated atherosclerosis in systemic lupus erythematosus. *Arthritis Rheum*. 2001;44:2331–2337.
20. Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. *Circulation*. 2002;105:1135–1143.
21. Ross R. Atherosclerosis–an inflammatory disease. *N Engl J Med*. 1999;340:115–126. doi: 10.1056/NEJM199901143400207.
22. van Leuven SI, Franssen R, Kastelein JJ, Levi M, Stroes ES, Tak PP. Systemic inflammation as a risk factor for atherothrombosis. *Rheumatology (Oxford)*. 2008;47:3–7. doi: 10.1093/rheumatology/kem202.
23. Bailey EL, Smith C, Sudlow CL, Wardlaw JM. Pathology of lacunar ischemic stroke in humans–a systematic review. *Brain Pathol*. 2012;22:583–591. doi: 10.1111/j.1750-3639.2012.00575.x.
24. Lammie GA. Pathology of small vessel stroke. *Br Med Bull*. 2000;56:296–306.
25. Moher D, Liberati A, Tetzlaff J, Altman DG; PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med*. 2009;6:e1000097. doi: 10.1371/journal.pmed.1000097.
26. Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. *JAMA*. 2000;283:2008–2012.
27. Wardlaw JM, Smith EE, Biessels GJ, Cordonnier C, Fazekas F, Frayne R, et al; STandards for ReportIng Vascular changes on nEuroimaging (STRIVE v1). Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration. *Lancet Neurol*. 2013;12:822–838. doi: 10.1016/S1474-4422(13)70124-8.
28. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP; Iniciativa STROBE. [The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies]. *Rev Esp Salud Publica*. 2008;82:251–259.
29. Bamford J, Sandercock P, Jones L, Warlow C. The natural history of lacunar infarction: the Oxfordshire Community Stroke Project. *Stroke*. 1987;18:545–551.
30. R Core Team. R: A Language and Environment for Statistical Computing. Vienna, Au: R Foundation for Statistical Computing. 2013.
31. Higgins J, Greens S. *Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 [updated March 2011]. The Cochrane Collaboration*; 2011. Available at: www.cochrane-handbook.org. Accessed March 28, 2015.
32. Hak AE, Karlson EW, Feskanich D, Stampfer MJ, Costenbader KH. Systemic lupus erythematosus and the risk of cardiovascular disease: results from the nurses' health study. *Arthritis Rheum*. 2009;61:1396–1402. doi: 10.1002/art.24537.
33. Krishnan E. Stroke subtypes among young patients with systemic lupus erythematosus. *Am J Med*. 2005;118:1415. doi: 10.1016/j.amjmed.2005.05.026.
34. Liou TH, Huang SW, Lin JW, Chang YS, Wu CW, Lin HW. Risk of stroke in patients with rheumatism: a nationwide longitudinal population-based study. *Sci Rep*. 2014;4:5110. doi: 10.1038/srep05110.
35. Horreau C, Pouplard C, Brenaut E, Barnetche T, Misery L, Cribier B, et al. Cardiovascular morbidity and mortality in psoriasis and psoriatic arthritis: a systematic literature review. *J Eur Acad Dermatol Venereol*. 2013;27 (Suppl 3):12–29. doi: 10.1111/jdv.12163.
36. Armstrong EJ, Harskamp CT, Armstrong AW. Psoriasis and major adverse cardiovascular events: a systematic review and meta-analysis of observational studies. *J Am Heart Assoc*. 2013;2:e000062. doi: 10.1161/JAHA.113.000062.
37. Brauchli YB, Jick SS, Miret M, Meier CR. Psoriasis and risk of incident myocardial infarction, stroke or transient ischaemic attack: an inception cohort study with a nested case-control analysis. *Br J Dermatol*. 2009;160:1048–1056. doi: 10.1111/j.1365-2133.2008.09020.x.
38. Dowlatshahi EA, Kavousi M, Nijsten T, Ikram MA, Hofman A, Franco OH, et al. Psoriasis is not associated with atherosclerosis and incident cardiovascular events: the Rotterdam Study. *J Invest Dermatol*. 2013;133:2347–2354. doi: 10.1038/jid.2013.131.
39. Gelfand JM, Dommasch ED, Shin DB, Azfar RS, Kurd SK, Wang X, et al. The risk of stroke in patients with psoriasis. *J Invest Dermatol*. 2009;129:2411–2418. doi: 10.1038/jid.2009.112.
40. Kaye JA, Li L, Jick SS. Incidence of risk factors for myocardial infarction and other vascular diseases in patients with psoriasis. *Br J Dermatol*. 2008;159:895–902. doi: 10.1111/j.1365-2133.2008.08707.x.
41. Kimball AB, Robinson D Jr, Wu Y, Guzzo C, Yeilding N, Paramore C, et al. Cardiovascular disease and risk factors among psoriasis patients in two US healthcare databases, 2001-2002. *Dermatology*. 2008;217:27–37. doi: 10.1159/000121333.
42. Li WQ, Han JL, Manson JE, Rimm EB, Rexrode KM, Curhan GC, et al. Psoriasis and risk of nonfatal cardiovascular disease in U.S. women: a cohort study. *Br J Dermatol*. 2012;166:811–818. doi: 10.1111/j.1365-2133.2011.10774.x.
43. Ogdie A, Yu Y, Haynes K, Love TJ, Maliha S, Jiang Y, et al. Risk of major cardiovascular events in patients with psoriatic arthritis, psoriasis and rheumatoid arthritis: a population-based cohort study. *Ann Rheum Dis*. 2015;74:326–332. doi: 10.1136/annrheumdis-2014-205675.
44. Schmitt J, Ford DE. Psoriasis is independently associated with psychiatric morbidity and adverse cardiovascular risk factors, but not with cardiovascular events in a population-based sample. *J Eur Acad Dermatol Venereol*. 2010;24:885–892. doi: 10.1111/j.1468-3083.2009.03537.x.
45. Yang YW, Keller JJ, Lin HC. Medical comorbidity associated with psoriasis in adults: a population-based study. *Br J Dermatol*. 2011;165:1037–1043. doi: 10.1111/j.1365-2133.2011.10494.x.
46. Han C, Robinson DW Jr, Hackett MV, Paramore LC, Fraeman KH, Bala MV. Cardiovascular disease and risk factors in patients with rheumatoid arthritis, psoriatic arthritis, and ankylosing spondylitis. *J Rheumatol*. 2006;33:2167–2172.
47. Meune C, Touzé E, Trinquart L, Allanore Y. High risk of clinical cardiovascular events in rheumatoid arthritis: levels of associations of myocardial infarction and stroke through a systematic review and meta-analysis. *Arch Cardiovasc Dis*. 2010;103:253–261. doi: 10.1016/j.acvd.2010.03.007.
48. Rahman MM, Kopec JA, Cibere J, Goldsmith CH, Anis AH. The relationship between osteoarthritis and cardiovascular disease in a population health survey: a cross-sectional study. *BMJ Open*. 2013;3. doi: 10.1136/bmjopen-2013-002624.
49. Watson DJ, Rhodes T, Guess HA. All-cause mortality and vascular events among patients with rheumatoid arthritis, osteoarthritis, or no arthritis in the UK General Practice Research Database. *J Rheumatol*. 2003;30:1196–1202.
50. Mikdashi J, Handwerger B, Langenberg P, Miller M, Kittner S. Baseline disease activity, hyperlipidemia, and hypertension are predictive factors for ischemic stroke and stroke severity in systemic lupus erythematosus. *Stroke*. 2007;38:281–285. doi: 10.1161/01.STR.0000254476.05620.14.
51. Low AS, Lunt M, Mercer, LK, Galloway JB, Davies R, Watson, KD, et al. Association between anti-tumor necrosis factor therapy and the risk of ischemic stroke in subjects with rheumatoid arthritis. Results from the British Society for Rheumatology Biologics Registers-Rheumatoid Arthritis (BSRBR-RA) [abstract]. *Arthritis Rheum*. 2012:64.
52. Zöller B, Li X, Sundquist J, Sundquist K. Risk of subsequent ischemic and hemorrhagic stroke in patients hospitalized for immune-mediated diseases: a nationwide follow-up study from Sweden. *BMC Neurol*. 2012;12:41. doi: 10.1186/1471-2377-12-41.
53. Turesson C, Jarenros A, Jacobsson L. Increased incidence of cardiovascular disease in patients with rheumatoid arthritis: results from a community based study. *Ann Rheum Dis*. 2004;63:952–955. doi: 10.1136/ard.2003.018101.
54. Semb AG, Kvien TK, Aastveit AH, Jungner I, Pedersen TR, Walldius G, et al. Lipids, myocardial infarction and ischaemic stroke in patients with rheumatoid arthritis in the Apolipoprotein-related Mortality RISk (AMORIS) Study. *Ann Rheum Dis*. 2010;69:1996–2001. doi: 10.1136/ard.2009.126128.
55. Nadarajahvili Z, Michaud K, Hallenbeck JM, Wolfe F. Cardiovascular, rheumatologic, and pharmacologic predictors of stroke in patients with rheumatoid arthritis: a nested, case-control study. *Arthritis Rheum*. 2008;59:1090–1096. doi: 10.1002/art.23935.

56. Gustafsson J, Gunnarsson I, Borjesson O, Pettersson S, Möller S, Fei GZ, et al. Predictors of the first cardiovascular event in patients with systemic lupus erythematosus—a prospective cohort study. *Arthritis Res Ther*. 2009;11:R186. doi: 10.1186/ar2878.
57. Chiu CC, Huang CC, Chan WL, Chung CM, Huang PH, Lin SJ, et al. Increased risk of ischemic stroke in patients with systemic lupus erythematosus: a nationwide population-based study. *Intern Med*. 2012;51:17–21.
58. Rothwell PM, Coull AJ, Silver LE, Fairhead JF, Giles MF, Lovelock CE, et al; Oxford Vascular Study. Population-based study of event-rate, incidence, case fatality, and mortality for all acute vascular events in all arterial territories (Oxford Vascular Study). *Lancet*. 2005;366:1773–1783. doi: 10.1016/S0140-6736(05)67702-1.
59. Sacco S, Marini C, Totaro R, Russo T, Cerone D, Carolei A. A population-based study of the incidence and prognosis of lacunar stroke. *Neurology*. 2006;66:1335–1338. doi: 10.1212/01.wnl.0000210457.89798.0e.
60. Ahlehoff O, Gislason GH, Charlot M, Jørgensen CH, Lindhardsen J, Olesen JB, et al. Psoriasis is associated with clinically significant cardiovascular risk: a Danish nationwide cohort study. *J Intern Med*. 2011;270:147–157. doi: 10.1111/j.1365-2796.2010.02310.x.
61. Holmqvist M, Simard JF, Asplund K, Arkema EV. Stroke in systemic lupus erythematosus: a meta-analysis of population-based cohort studies. *RMD Open*. 2015;1:e000168. doi: 10.1136/rmdopen-2015-000168.
62. Sudlow CL, Warlow CP. Comparing stroke incidence worldwide: what makes studies comparable? *Stroke*. 1996;27:550–558.
63. Bos MJ, Koudstaal PJ, Hofman A, Witteman JC, Breteler MM. Uric acid is a risk factor for myocardial infarction and stroke: the Rotterdam study. *Stroke*. 2006;37:1503–1507. doi: 10.1161/01.STR.0000221716.55088.d4.
64. Lindsberg PJ, Grau AJ. Inflammation and infections as risk factors for ischemic stroke. *Stroke*. 2003;34:2518–2532. doi: 10.1161/01.STR.0000089015.51603.CC.
65. Wiseman S, Marlborough F, Doubal F, Webb DJ, Wardlaw J. Blood markers of coagulation, fibrinolysis, endothelial dysfunction and inflammation in lacunar stroke versus non-lacunar stroke and non-stroke: systematic review and meta-analysis. *Cerebrovasc Dis*. 2014;37:64–75. doi: 10.1159/000356789.
66. Kaichi Y, Kakeda S, Moriya J, Ohnari N, Saito K, Tanaka Y, et al. Brain MR findings in patients with systemic lupus erythematosus with and without antiphospholipid antibody syndrome. *AJNR Am J Neuroradiol*. 2014;35:100–105. doi: 10.3174/ajnr.A3645.
67. Appenzeller S, Vasconcelos Faria A, Li LM, Costallat LT, Cendes F. Quantitative magnetic resonance imaging analyses and clinical significance of hyperintense white matter lesions in systemic lupus erythematosus patients. *Ann Neurol*. 2008;64:635–643. doi: 10.1002/ana.21483.
68. Valdés-Ferrer SI, Vega F, Cantú-Brito C, Ceballos-Ceballos J, Estañol B, García-Ramos G, et al. Cerebral changes in SLE with or without antiphospholipid syndrome. a case-control MRI study. *J Neuroimaging*. 2008;18:62–65. doi: 10.1111/j.1552-6569.2007.00183.x.
69. Debette S, Markus HS. The clinical importance of white matter hyperintensities on brain magnetic resonance imaging: systematic review and meta-analysis. *BMJ*. 2010;341:c3666.

Appendix B: Letter from Lupus UK awarding study funds

Institute of Fundraising

FundRaising Standards Board
give with confidence

LUPUS UK

"Caring for people with lupus"

Professor Joanna M Wardlaw
Neuroimaging Sciences
University of Edinburgh
Western General Hospital
Crewe Road
Edinburgh
EH4 2XU

8th November 2013

Dear Professor Wardlaw

Re: Pilot grant application, "A pilot magnetic resonance neuroimaging study of patients with lupus"

The LUPUS UK Peer Review Panel have made their recommendation to the Trustees of LUPUS UK and I am pleased to advise that they have agreed funding for this project for the sum requested of £24,440, subject to ethics approval. In addition the Panel have recommended that BILAG is used for assessment and APL antibodies and hormone status should be included.

Also can you please confirm the source of the other funding for the project, total cost £144,124.

If you have any queries relating to this decision please let me know and I will pass them on to the Panel.

Subject to the above, please advise your finance department that the funding for this research project will be payable against four quarterly invoices of £6,110.

Yours sincerely

Chris Maker
Director

LUPUS UK Visit our website: www.lupusuk.org.uk
Reg Charity Nos. 1051610, SC039682
National Office:
St James House, Eastern Road, Romford, Essex, RM1 3NH
Tel: 01708 731251
Director: Chris Maker ACIB

Appendix C: Clinical criteria for diagnosing SLE

Table 2. The 1982 revised criteria for classification of systemic lupus erythematosus*

Criterion		Definition
1. Malar rash		Fixed erythema, flat or raised, over the malar eminences, tending to spare the nasolabial folds
2. Discoid rash		Erythematous raised patches with adherent keratotic scaling and follicular plugging; atrophic scarring may occur in older lesions
3. Photosensitivity		Skin rash as a result of unusual reaction to sunlight, by patient history or physician observation
4. Oral ulcers		Oral or nasopharyngeal ulceration, usually painless, observed by a physician
5. Arthritis		Nonerosive arthritis involving 2 or more peripheral joints, characterized by tenderness, swelling, or effusion
6. Serositis	a)	Pleuritis—convincing history of pleuritic pain or rub heard by a physician or evidence of pleural effusion *OR*
	b)	Pericarditis—documented by ECG or rub or evidence of pericardial effusion
7. Renal disorder	a)	Persistent proteinuria greater than 0.5 grams per day or greater than 3+ if quantitation not peformed *OR*
	b)	Cellular casts—may be red cell, hemoglobin, granular, tubular, or mixed
8.Neurologic disorder	a)	Seizures—in the absence of offending drugs or known metabolic derangements; e.g., uremia, ketoacidosis, or electrolyte imbalance *OR*
	b)	Psychosis—in the absence of offending drugs or known metabolic derangements, e.g., uremia, ketoacidosis, or electrolyte imbalance
9. Hematologic disorder	a)	Hemolytic anemia—with reticulocytosis *OR*
	b)	Leukopenia—less than 4,000/mm^3 total on 2 or more occasions *OR*
	c)	Lymphopenia—less than 1,500/mm^3 on 2 or more occasions *OR*
	d)	Thrombocytopenia—less than 100,000/mm^3 in the absence of offending drugs
10. Immunologic disorder	a)	Positive LE cell preparation *OR*
	b)	Anti-DNA: antibody to native DNA in abnormal titer *OR*
	c)	Anti-Sm: presence of antibody to Sm nuclear antigen *OR*
	d)	False positive serologic test for syphilis known to be positive for at least 6 months and confirmed by *Treponema pallidum* immobilization or fluorescent treponemal antibody absorption test
11. Antinuclear antibody		An abnormal titer of antinuclear antibody by immunofluorescence or an equivalent assay at any point in time and in the absence of drugs known to be associated with "drug-induced lupus" syndrome

* The proposed classification is based on 11 criteria. For the purpose of identifying patients in clinical studies, a person shall be said to have systemic lupus erythematosus if any 4 or more of the 11 criteria are present, serially or simultaneously, during any interval of observation.

Appendix D: Approval from SE Scotland Research Ethics Committee

Lothian NHS Board

South East Scotland Research Ethics Committee 01

Waverley Gate
2-4 Waterloo Place
Edinburgh
EH1 3EG
Telephone 0131 536 9000
Fax 0131 465 5789

www.nhslothian.scot.nhs.uk

Date 02 February 2014
Your Ref
Our Ref

Enquiries to: Sandra Wyllie
Extension: 35473
Direct Line: 0131 465 5473
Email: Sandra.Wyllie@nhslothian.scot.nhs.uk

Professor Joanna Wardlaw
Professor of Neuroimaging
University of Edinburgh
Department of Clinical Neurosciences
Western General Hospital, Crewe Road
EH4 2XU

Dear Professor Wardlaw

Study title: **A single-site, pilot, prospective, observational, magnetic resonance neuroimaging study of patients with the inflammatory rheumatic disorder lupus for signs of cerebral small vessel disease and associations with cognition, fatigue and depression and blood markers of inflammation and lupus disease activity.**

REC reference: **14/SS/0003**

IRAS project ID: **135313**

Thank you for your letter of 30 January 2014, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details, unless you expressly withhold permission to do so. Publication will be no earlier than three months from the date of this favourable opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to withhold permission to publish, please contact the REC Manager Mrs Sandra Wyllie, Sandra.Wyllie@nhslothian.scot.nhs.uk.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

NHS
Lothian

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database within 6 weeks of recruitment of the first participant (for medical device studies, within the timeline determined by the current registration and publication trees).

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non clinical trials this is not currently mandatory.

If a sponsor wishes to contest the need for registration they should contact Catherine Blewett (catherineblewett@nhs.net), the HRA does not, however, expect exceptions to be made. Guidance on where to register is provided within IRAS.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Advertisement	Version 1	02 December 2013
GP/Consultant Information Sheets	Version 1- Letter to GP	18 November 2013
GP/Consultant Information Sheets	Version 1- Letter to Rheumatologists	21 November 2013
Other: Permission to use Fatigue Severity Scale		
Other: LIPUS award letter		08 November 2013

Other: Permission to use MoCA letter		14 November 2013
Other: CV - CI - Prof J Wardlaw		
Other: CV - Student - S Wiseman		
Other: Letter of invite - given at clinic	Version 1	18 November 2013
Other: Letter of invite - sent in advance	Version 1	26 October 2013
Other: LIST OF RHEUMATOLOGISTS	Version 1	27 November 2013
Participant Consent Form	Version 1	17 November 2013
Participant Information Sheet	Version 2	20 January 2014
Protocol	Version 1	31 October 2013
Questionnaire: Hospital Anxiety and Depression Scale (HADS)		
Questionnaire: Montreal Cognitive Assessment (MoCA)		
Questionnaire: Addenbrookes Cognitive Examination-R (ACE-III)		
Questionnaire: Mini Mental State Examination (MMSE)		
Questionnaire: National Adult Reading Test (NART)		
Questionnaire: British Isles Lupus Assessment Group Index (BILAG)		
Questionnaire: SLE Disease Activity Index (SLEDAI)		
Questionnaire: Systemic Lupus International Collaborating Clinics Damage Index		
Questionnaire: Fatigue Severity Scale (FSS)		
REC application		24 December 2013
Response to Request for Further Information		30 January 2014

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document *"After ethical review – guidance for researchers"* gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

Feedback

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

NHS
Lothian

Further information is available at National Research Ethics Service website > After Review

14/SS/0003	Please quote this number on all correspondence

We are pleased to welcome researchers and R & D staff at our NRES committee members' training days – see details at http://www.hra.nhs.uk/hra-training/

With the Committee's best wishes for the success of this project.

Yours sincerely

Dr Janet Andrews
Chair

Email:Sandra.Wyllie@nhslothian.scot.nhs.uk

Enclosures: "After ethical review – guidance for researchers"

Copy to: *Marianne Laird*
Karen Maitland, NHS Lothian

Appendix E: Approval from NHS Lothian R&D

University Hospitals Division

NHS Lothian

Queen's Medical Research Institute
47 Little France Crescent, Edinburgh, EH16 4TJ

FM/TM/approval

4 February 2014

Research & Development
Room E1.12
Tel: 0131 242 3330
Fax: 0131 242 3343

Email:
R&DOffice@nhslothian.scot.nhs.uk

Director: Professor David E Newby

Mr Stewart Wiseman
University of Edinburgh
Division of Clinical Neurosciences
Western General Hospital
Crewe Road
Edinburgh
EH4 2XU

Dear Mr Wiseman

Lothian R&D Project No: 2014/0007

Title of Research: A single-site, pilot, prospective, observational, magnetic resonance neuroimaging study of patients with the inflammatory rheumatic disorder lupus for signs of cerebral small vessel disease and associations with cognition, fatigue and depression and blood markers of inflammation and lupus disease activity

REC No: 14/SS/0003

Patient Information Sheet:
version 2 dated 20 January 2014

Consent Form:
version 1 dated 17 November 2013

Protocol: version 1 dated 31 October 2013

I am pleased to inform you that this study has been approved for NHS Lothian and you may proceed with your research, subject to the conditions below. This letter provides Site Specific approval for **NHS Lothian.**

Please note that the NHS Lothian R&D Office must be informed if there are any changes to the study such as amendments to the protocol, recruitment, funding, personnel or resource input required of NHS Lothian. This includes any changes made subsequent to management approval and prior to favourable opinion from the REC

Substantial amendments to the protocol will require approval from the ethics committee which approved your study and the MHRA where applicable

Please inform this office when recruitment has closed and when the study has been completed

I wish you every success with your study.

Yours sincerely

Fiona McArdle.

Ms Fiona McArdle
Deputy R&D Director

STA034

cc Joanna Wardlaw, Chief Investigator
Duncan Martin, BRIC
Dawn Lyster, Labs
Stuart Ralston, Head of Service
Sharon Cameron, CRF
Paul Dearie, QA Manager
Karen Maitland, Research Governance Manager

Appendix F: Protocol

Neuroimaging Sciences
University of Edinburgh
DCN
Western General Hospital
Crewe Road
Edinburgh EH4 2XU

Principal Investigator: Stewart Wiseman swiseman@staffmail.ed.ac.uk Tel: 0131 537 2660 or 0131 537 1985
Chief Investigator: Professor Joanna Wardlaw

Pilot MRI scanning study of the brains of patients with lupus

Study Protocol (single-site) E131321

Title: A single-site, pilot, prospective, observational, magnetic resonance neuroimaging study of patients with the inflammatory rheumatic disorder lupus for signs of cerebral small vessel disease and associations with cognition, fatigue and depression and blood markers of inflammation and lupus disease activity.

Short title: Pilot MRI scanning study of the brains of patients with lupus

Co-sponsors	University of Edinburgh & NHS Lothian ACCORD The Queen's Medical Research Institute 47 Little France Crescent Edinburgh EH16 4TJ
Funder	University of Edinburgh and Lupus UK
Chief Investigator	Professor Joanna Wardlaw
REC Number	14/SS/0003
Version Number and Date	See footer

CONTENTS

LIST OF ABBREVIATIONS

ACCORD	Academic and Clinical Central Office for Research & Development - Joint office for University of Edinburgh and NHS Lothian
CRF	Case Report Form
DTI	Diffusion tensor imaging (a type of MRI scanning sequence)
EPVS, PVS	Enlarged perivascular spaces
GCP	Good Clinical Practice
FLAIR	Fluid Attenuated Inversion Recovery (a type of MRI scanning sequence)
GRE	Gradient recalled echo (a type of MRI scanning sequence)
ICH	International Conference on Harmonisation
MRI	Magnetic resonance imaging
REC	Research Ethics Committee
SLE	Systemic Lupus Erythematosus
SLEx	Scottish Lupus Exchange Database
SOP	Standard Operating Procedure
SVD	(Cerebral) small vessel disease
WMH	White matter hyperintensities
WTCRF	Wellcome Trust Clinical Research Facility

WHO IS RUNNING THE STUDY / DUTIES

Study Team

Stewart Wiseman – Principal Investigator

Radiographer and PhD candidate.

Duties:

- Receive informed consent; telephone contact with participants; scanning; administration of cognitive and other mental status tests; blood pressure readings; data collection and entry onto Case Record Form; analysis; archiving; data guardian

Nicole Amft – Clinical Collaborator

Consultant Rheumatologist.

Duties:

- Referrer; assess if potential recruits meet inclusion/exclusion criteria; clinician responsible for the medical care of recruits

Veena Dhillon, Michael Lambert, Neil McKay, Euan McRorie, Philip Riches, Stuart Ralston

Consultant Rheumatologists

Duties:

- Referrers; assess if potential recruits meet inclusion/exclusion criteria; clinicians responsible for the medical care of recruits; will make first approach to their patients

Mark Bastin

Medical Physicist – MR Imaging

Duties:

- Analysis and oversight of tractography scan data

Kirsten Schuler

Administrator

Duties:

- Data administration

Francessca Chappell

Statistician

Duties:

- Statistical advice – design and analysis

Joanna Wardlaw – Chief Investigator & Academic Supervisor

Professor of Neuroimaging and Honorary Consultant Neuroradiologist

Duties:

- Overall responsibility, study oversight and academic guidance

Stuart Ralston – Academic Supervisor

Professor of Rheumatology and Honorary Consultant Rheumatologist

Duties:

- Academic guidance

Elaine Sandeman, Iona Hamilton, Gayle Barclay, Charlotte Sutherland

Radiographers

Duties:

- Scanning; administration of cognitive and other mental status tests; blood pressure reading

Caroline Patterson (University of Dundee)

Lupus UK rheumatology nurse

Duties:

- Main Scottish Lupus Exchange Database collaborator

Wellcome Trust Clinical Research Facility nurses

Duties:

- Venepuncture and blood collection

Scottish Lupus Exchange Database Participants

Professor Jill Belch and Dr Stephen McSwiggan from the University of Dundee, on behalf of the SLEx group.

Duties:

- Strategic input; data collaboration

Support Services – Authorisations

Stuart Ralston

- to authorise use of NHS Lothian rheumatology service

Dawn Lyster (NHS Lothian)

- to authorise use of NHS Lothian labs for blood analysis

Sharon Cameron (Deputy Director)

- to authorise use of WTCRF for venepuncture, blood processing and storage

Duncan Martin (Business Manager)

- to authorise use of BRIC for imaging, data analysis, database management, archiving.

SUMMARY

Quick Scientific Summary
This is a single-site pilot neuroimaging project to see if patients with the inflammatory rheumatic disease lupus have evidence of cerebral small vessel disease (SVD) on structural MRI and/or evidence of sub-visible white matter damage on advanced tractography MRI scans.

Our primary objective is to test our methods and procedures and to collaborate with the Scottish Lupus Exchange, a new national lupus registry project being led from the University of Dundee and which is currently recruiting form across Scotland, including Edinburgh. This proposed brain scanning project will recruit from Edinburgh only, but where a patient is also participating in the SLEx database, we will seek to share data to lessen the burden on patients. There is potential for a larger multi-centre imaging project in the future.

Our main research question is to determine if various brain imaging features of cerebral SVD (white matter hyperintensities, enlarged perivascular spaces, lacunes, acute small subcortical infarcts, microbleeds and whole brain shrinkage) are present in patients with lupus, and if so, are the MR imaging features associated with (a) fatigue, depression and cognitive impairment on formal testing and (b) blood markers of inflammation, measures of lupus disease activity, specific antibodies, hormones and accumulated rheumatic damage.

Our secondary objectives are to see if there is a difference in various brain imaging features of cerebral SVD between our lupus group and (a) historical controls of patients with mild stroke scanned on the same scanner, matched on age, sex and vascular risk factors and (b) to compare quantitative tractography measures of sub-visible white matter damage (FA and MD values per tract) with historical healthy controls scanned on the same scanner, matched on age and sex.

Lay Summary
Patients with rheumatic diseases such as lupus have an increased risk of stroke compared to the general population. Lupus patients also suffer other kinds of neurological problems that add to loss of wellbeing such as headache, cognitive difficulties, fatigue, mood disorders, memory problems, seizures and problems with movement and speech. These can be mild, severe or fatal. The causes are not well understood, so treatment of these symptoms is poor. Detailed magnetic resonance (MR) brain scanning can help to understand how lupus is affecting the blood vessels that supply the brain and the brain itself, and find possible ways of monitoring and preventing the damage from accumulating. However most studies so far that used scanning, including some that included many patients, only scanned patients with symptoms, did not use very detailed scanning, did not compare the lupus patients with healthy controls, or make adjustments for common things like high blood pressure that we know can also affect the brain.

We have developed very detailed, non-invasive MR scanning methods to measure brain abnormalities that we know affect cognition, mood, memory, movement and increase stroke risk in other diseases such as cerebral small vessel disease which is a major cause of stroke, cognitive decline and physical impairments. We can also detect subtle changes in the brain before obvious abnormalities show up on routine scanning. These MR techniques are the result of our interest in stroke, age-related cognitive decline and dementia. The type of stroke caused by small vessel disease, lacunar stroke, affects people at younger ages, causes small lesions deep in the brain, and can result in cognitive impairment, gait disturbance and mood disorders. Commonly these patients have other asymptomatic

lesions in the brain that increase the risk of dementia and stroke. There are some similarities between this type of stroke and the limited information that we have about lupus patients, suggesting that some of the cause of this type of small stroke may also be relevant to understanding what causes the brain damage in lupus.

Lupus patients often feel frustrated that the symptoms which seem to affect the brain are poorly understood and not very well treated. Neurological symptoms in lupus are common, but variable, and a specific diagnosis of neurolupus is difficult to arrive at, and comes with challenges regarding treatment choices. Routine brain imaging can be 'normal' or 'non-specific' which adds to the frustration. Neurolupus could be an advanced stage of something happening in all lupus patients. It is possible that lupus patients who do not have noticeable neurological or cognitive symptoms have "early subclinical" cognitive changes that can be detected on formal cognitive testing or changes in their brains that can be detected with advanced MR techniques.

This proposal is for a pilot project to use MR brain imaging to see what changes are present in the brain in patients with lupus, regardless of whether they have brain symptoms or not, to see if these are related to symptoms of fatigue, depression, cognitive difficulties or evidence of these difficulties on formal testing, and to see if the brain imaging features relate to lupus disease activity.

We will compare the scans from the lupus patients with scans and other medical information from our studies of patients with small vessel stroke and normal volunteers of similar ages, scanned on the same scanner. Before we can embark on a large national project, we need to find out how common different findings might be to plan a larger study, test if the patients are happy with the scans and do not mind the cognitive testing, and check that our methods of analysing the images work in scans of patients with lupus.

The investigators have considerable expertise in imaging of brain vascular disease, and in assessment and understanding of inflammatory diseases including lupus. We will work with the Scottish Lupus Exchange (SLEx) Database lead by Prof Jill Belch, Dundee, that is already assessing patients attending lupus clinics in Scotland, including the clinic in Edinburgh to reduce the burden of participation for patients. Working together and sharing information will cut down on the information or blood samples that we need to collect from the patients. We are working with the NHS Lothian Rheumatology Service's audit of rheumatology patients. The research scanning facility is all set up, we have the right analysis methods and staff to recruit the patients and perform the analysis.

If successful, we plan to extend the study to perform brain scanning in patients attending regional lupus clinics in Dundee, Aberdeen and Glasgow, all of which have scanners set up to run the same imaging through our collaborative imaging network, the Scottish Imaging Network, A Platform for Scientific Excellence (SINAPSE, www.sinapse.ac.uk). Funding from a larger charity would be sought for this, in collaboration with the SLEx Investigator Group. The results will also help to understand how inflammation might affect the brain in patients with small vessel stroke and we plan to apply for funding for a larger study from relevant stroke research funding organisations.

Scientific Summary

Introduction

Patients with chronic rheumatic diseases including lupus have a dysfunctional, non-resolving immune response, with multi-organ involvement including the kidneys and brain. Patients with rheumatic disease are at increased risk of cardiovascular events including stroke. At least seven studies (totalling some 39,630 patients with lupus) show an increased incidence of stroke in patients with lupus, with age and sex-matched hazard ratios of between 1.5 and 2.0.[1–7] Most of these strokes are described as ischemic but there is little information on the subtype of ischemic stroke.

Lupus patients also complain of fatigue, 'brain fog', cognitive impairment and other neurological involvement.[8] At least three prospective studies (n=1,385) document rates of at least one episode of psychiatric disturbance over several years of around 40%, as well as headaches, cognitive disturbance and neurological symptoms that fall short of stroke.[9–11] These symptoms are disabling, unpleasant and contribute substantially to the burden of disease. At least 19 studies (average n=30) using MR imaging suggest that patients with lupus are more likely to have brain shrinkage or hyperintense lesions in the white matter (WMH), but the studies vary in their estimates of abnormalities, are generally small, most focus only on patients with symptoms, many do not include controls or focus on only one abnormality (rather than assessing all related abnormalities) and many do not correct for vascular risk factors. Therefore it is difficult to determine how common these various abnormalities are and whether they are truly associated with or potentially causative of these symptoms. Finally, brain pathology studies in lupus, including two with some post-mortem MR brain imaging, describe microinfarcts, small vessel vasculopathy, cortical atrophy, and ischemic demyelination.[12 (Cohen in submission)]

These pathology features in lupus, and some of the neurological symptoms, are similar to those seen in cerebral small vessel disease (SVD).[13–17] SVD causes one subtype of stroke (lacunar stroke, which is usually ischaemic but can sometimes be haemorrhagic) as well as diffuse hyperintense abnormalities in the brain white matter, microhaemorrhages, brain atrophy and sub-visible abnormalities detectable with methods like MR diffusion tensor imaging.[18] The cause of SVD is also unknown but also appears to involve inflammation in the small vessels on pathology.[13] SVD is important, because lacunar stroke accounts for 25% of all strokes and the affected patients are often only in their 40s or 50s. SVD also increases the risk of recurrent stroke and dementia, being responsible wholly or in part for about 45% of all ageing-related dementias.[19] Various pathogenic processes have been implicated, of which endothelial dysfunction, possibly exacerbated by inflammation which damages the blood-brain barrier further, has the most evidence.[18]

This pilot study is part of work to improve understanding of how inflammation affects the brain vessels and tissue and relates to symptoms, with the aim ultimately of improving prevention and treatment. The detection of early changes in the brain could influence the management of patients with lupus to prevent disabling neurological symptoms and progressive brain damage. This knowledge will also contribute to understanding of how inflammation affects the brain to improve prevention and treatment of SVD-related lacunar stroke, cognitive decline and dementia.

Literature critique

The following is from our systematic review of stroke and neuroimaging findings in lupus

which is ongoing. Neuroimaging studies in lupus have been performed since the 1980s. Numerous studies as noted above report more atrophy and white matter hyperintensities in patients with lupus than in controls, but most are small and do not control for confounders.[20,21] Several studies[9,21–26] dichotomise lupus patients into "lupus" versus "neuropsychiatric lupus" and found more abnormalities on MR imaging in those with neuropsychiatric events. Sub-visible changes were also found in two small studies (n=34[27] and 8[28]) with MR diffusion tensor imaging (higher whole brain diffusion constants and lower regional fractional anisotropy) in all lupus patients, even those with a "normal" routine MR scan, versus non-lupus controls. Thus, neurolupus might represent an advanced presentation of a process underway in all lupus patients, detectable by routine MR only when sufficiently advanced or during a period of active flare. Whether neurolupus is a consequence of more severe lupus activity or occurs in patients whose brains are more vulnerable to the effects of inflammation and white matter damage, is unclear, but this distinction would have important implications for prevention and treatment of neurological complications of lupus.

Pathology studies[29] support MR imaging findings. In a post mortem immunohistochemistry study of 34 lupus patients and 24 controls (dying of ischemic heart disease):[12] microinfarction (p=0.016), macroinfarction (p=0.002), vasculitis (p=0.048) and diffuse vasculopathy (p=0.001) were more often present in neurolupus (n=16) than in non-neurolupus (n=18); focal vasculopathy was present in 40-60% of all lupus subjects. Complement deposition (C1q and C4d) was observed on endothelial cells of small arterioles in lupus and neurolupus, significantly more so than controls (p=0.001), but showed no difference between lupus and neurolupus.

In a prospective study of 337 lupus patients from 1987 to 1995 to assess homocysteine as a risk factor for stroke in lupus,[30] there were 29 strokes (8.6%) and homocysteine was raised in 15% (51/337) of lupus patients (odds ratio, OR, 2.44 95% CI 1.04-5.75 adjusted for vascular risk factors). Our[31] recent systematic review and meta-analysis of blood markers in stroke (accepted, in production) showed homocysteine to be significantly higher in lacunar stroke versus non-stroke controls (SMD 0.55, 95% CI 0.42-0.69).

Many of these studies describe features on MR imaging that are very similar to features of ageing and stroke-associated cerebral SVD such as WMH, lacunes and atrophy. Furthermore, WMH which were previously thought to be silent accompaniments of ageing and of no clinical consequence, are now recognised to double the risk of dementia, treble the risk of stroke and cause substantial physical disabilities (impaired balance, gait and bladder disturbance).[19,32] They are also not 'silent' but are now known to cause subjective awareness of cognitive impairment and subtle physical symptoms that fall short of stroke.[15–17]

Figure 1 illustrates some of these features and imaging methods that we use in their detection in a subject with ageing-related small vessel disease features on imaging. Perivascular inflammation around the brain perforating arterioles is seen on pathology, although its potential role has been under-recognised, the focus so far being on ischemic damage. However neuroimaging studies and experimental models have demonstrated a key role for blood-brain barrier disruption and leakage of fluid into the vessel walls and perivascular tissues leading to chronic arteriolar and perivascular brain damage manifesting as lacunar stroke, lacunes, WMH, atrophy and prominent perivascular spaces.[18] Lacunar stroke is mostly not atheromatous:[33] a recent trial showed that dual antiplatelet drugs (widely used for atheromatous stroke) increased haemorrhage and death without preventing recurrent lacunar stroke.[34,35] There is not much evidence for ischemia as an initiating step

although it may be a late secondary consequence of disrupted arterioles. Further confirmatory studies are ongoing, but the demonstration that SVD is not primarily ischemic or atheromatous is important for identifying treatment and prevention strategies. Emerging evidence also suggests that development of SVD reflects patients' vulnerability to arteriolar endothelial and white matter damage, rather than simply their vascular risk factor exposure.[36] The parallels with the neurological complications of lupus are striking, and suggest that knowledge of SVD can improve our understanding of how lupus affects the brain and new therapeutic approaches, and vice versa.

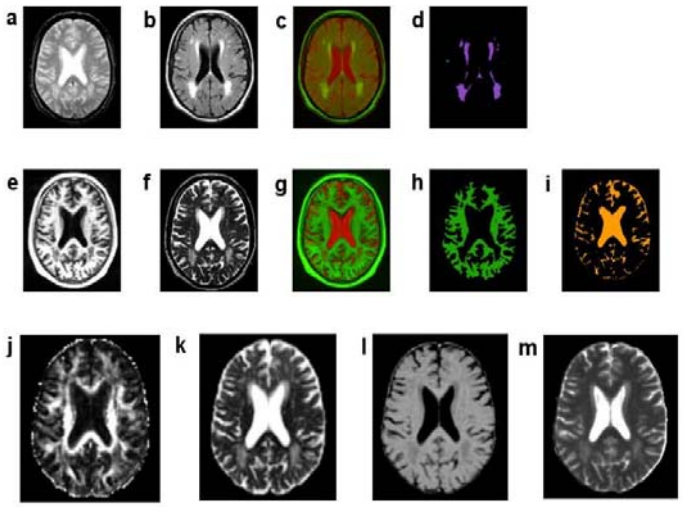


Figure 1 Typical multi-modal MR images from a subject with visible white matter abnormalities (WMH) in small vessel disease showing different visible and subvisible changes of SVD and how they can be detected and measured on MR imaging. $T2^*$-weighted (a) and FLAIR (fluid-attenuated inversion recovery) (b) structural scans are combined in the red-green colour space (c) to facilitate the extraction of image voxels corresponding to WMH (d). T1-weighted (e) and T2-weighted (f) structural scans are combined in the red-green colour space (g) to facilitate the extraction of the voxels corresponding to normal appearing white matter (NAWM) (h) and cerebrospinal fluid (i); the latter is subtracted from the WMH and NAWM masks. The last row shows reconstructed parametric images of MRI biomarkers: FA (j), MD (k) from diffusion tensor imaging, MTR (l) from magnetisation transfer imaging and T1 relaxation time (m) which provide information about subvisible changes in white matter integrity, myelin damage and water content respectively.

1 INTRODUCTION

1.1 BACKGROUND

Patients with lupus have an increased risk of stroke compared to the general population. At least seven studies (totalling some 39,630 patients with lupus) show an increased incidence of stroke in patients with lupus, with age and sex-matched hazard ratios of between 1.5 and 2.0.[1–7] Most of these strokes are described as ischemic but there is little information on the subtype of ischemic stroke.

Lupus patients also complain of fatigue, 'brain fog', cognitive impairment and other neurological involvement.[8] At least three prospective studies (n=1,385) document rates of at least one episode of psychiatric disturbance over several years of around 40%, as well as headaches, cognitive disturbance and neurological symptoms that fall short of stroke.[9–11] These symptoms are disabling, unpleasant and contribute substantially to the burden of disease. At least 19 studies (average sample size, n=30) using MR imaging suggest that patients with lupus are more likely to have brain shrinkage or hyperintense lesions in the white matter (WMH), but the studies vary in their estimates of abnormalities, are generally small, most focus only on patients with symptoms, many do not include controls or focus on only one abnormality (rather than assessing all related abnormalities) and many do not correct for vascular risk factors.

We have developed detailed, non-invasive MR scanning methods to measure brain abnormalities that we know affect cognition, mood, memory, movement and increase stroke risk in other diseases such as cerebral small vessel disease (SVD). We can also detect subtle changes in the brain before obvious abnormalities show up on routine scanning. These MR techniques are the result of our interest in stroke, age-related cognitive decline and dementia. There are some similarities between SVD and the limited information that we have about lupus patients, suggesting that some of the cause of SVD may also be relevant to understanding what causes brain damage in lupus.

We will invite lupus patients attending the NHS Lothian rheumatology service at the Western General Hospital to have a brain scan as part of a pilot project to see if we can apply our knowledge of brain imaging features from SVD to this cohort. Participants will undergo cognitive testing and have a small sample of blood taken to measure inflammation and other markers.

1.2 RATIONALE FOR STUDY

There are no studies that assess comprehensively all features of visible and sub-visible small vessel and brain tissue damage in lupus using advanced MR imaging, that include all patients and not just those with symptoms, that compare brain imaging to measures of fatigue and cognitive impairment in all patients, or with markers of disease activity, and which also control for common confounding vascular risk factors.

Previous brain imaging studies in lupus have typically focussed on patients exhibiting neurological symptoms alone, rather than all lupus patients, and did not use very detailed MR imaging. Our research design is to include all eligible lupus patients, with or without current neurological symptoms, and use advanced MR techniques that are better able to detect 'subclinical' brain changes that might explain how the brain is affected by lupus.

We considered scanning only those with active neurological involvement but we did not want to exclude any lupus patient, particularly given that most lupus patients will have reported 'brain fog' or other more serious neurological problems at some stage during their disease. This view was supported when we discussed the project with lupus patients. Our aims are:

Overview

- To perform a prospective single-site pilot study using MR brain imaging in patients with lupus to assess for visible changes in brain structure and sub-visible damage
- To collaborate with the Scottish Lupus Exchange, a new national database for monitoring lupus disease progression and management

Analysis – Within the lupus scan cohort

- To determine if various brain imaging features of cerebral small vessel disease (SVD) are present in this pilot lupus cohort
- Are brain imaging features of SVD in this pilot lupus cohort associated with:
    - fatigue, depression and cognitive impairment
    - blood markers of coagulation/fibrinolysis, inflammation, endothelial dysfunction and measures of lupus disease activity
    - formally assessed measures of lupus disease activity and accumulated systemic rheumatic damage
- To predict brain imaging features of cerebral small vessel disease in this pilot lupus cohort from scores of cognition or levels of blood markers of inflammation using multiple linear regression, controlling for known vascular risk factors

Analysis – Versus historical controls

- To compare various brain imaging features of cerebral small vessel disease with historical controls of patients with mild stroke scanned on the same scanner, matched on age, sex and vascular risk factors
- To compare quantitative tractography measures (FA & MD) of sub-visible white matter damage in specific white matter tracts with historical healthy controls scanned on the same scanner, matched on age and sex

2 STUDY OBJECTIVES

2.1 OBJECTIVES

2.1.1 Primary Objective

This is a pilot neuroimaging project to see if patients with lupus have evidence of cerebral small vessel disease on structural MRI and/or evidence of sub-visible white matter damage on advanced tractography MR scans. Our primary objective is to test our methods and procedures, to collaborate with the Scottish Lupus Exchange, to determine if various brain imaging features of cerebral small vessel disease in this pilot lupus cohort are associated with fatigue, depression and cognitive impairment on formal testing, and to see if brain imaging features are associated with blood markers of inflammation, measures of lupus disease activity, and accumulated systemic rheumatic damage.

2.1.2 Secondary Objectives

Our secondary objectives are to compare brain image findings to historical control data (a) various brain imaging features of cerebral small vessel disease with historical controls of patients with mild stroke scanned on the same scanner, matched on age, sex and vascular risk factors and (b) quantitative measures of sub-visible white matter damage with historical healthy controls scanned on the same scanner, matched on age and sex.

2.1.3 Main research question

Do patients with lupus have MR imaging evidence of cerebral SVD? In patients with lupus, are various brain imaging features of cerebral SVD associated with (a) fatigue, depression and cognitive impairment and (b) blood markers of inflammation, measures of lupus disease activity, and accumulated systemic rheumatic damage.

2.1.4 Secondary research question

Is there a difference in various brain imaging features of cerebral SVD between patients with lupus and historical controls of patients with mild stroke and is there a difference in quantitative measures of sub-visible white matter damage between patients with lupus and historical healthy controls.

3 STUDY DESIGN

A pilot single-site magnetic resonance neuroimaging study with measures of cognition, fatigue, depression, blood markers and lupus disease activity at one time point (cross-section). The study is expected to take 12-15 months, and is planned to run as follows:

<table>
<tr><th>Jan 2014 – Feb 2014</th><th>March 2014 – Sept 2014</th><th>To Dec 2014 / Mar 2015</th></tr>
<tr><td>Setting up of the experiment</td><td colspan="2"></td></tr>
<tr><td rowspan="3"></td><td>Patient recruitment, scanning, testing and blood analysis</td><td></td></tr>
<tr><td colspan="2">Data analysis</td></tr>
<tr><td></td><td>Writing up and report</td></tr>
</table>

3.1 Number of visits and what the participant will do

Each participant will be seen only once. This single session will be held at the Brain Research Imaging Centre, Western General Hospital, Edinburgh and will last no more than

2 hours. Participants will undergo an MRI brain scan lasting about 50 minutes, cognitive testing and assessments of anxiety, depression and fatigue also lasting about 50 minutes. The participant will have a small amount of blood drawn by venepuncture (about 5 or 5.5 teaspoonfuls) and a blood pressure reading.

4 STUDY POPULATION

4.1 NUMBER OF PARTICIPANTS

Around 50 patients diagnosed with lupus will participate at one site. We will also use data from 100 historical controls from similar studies at our centre.

4.2 INCLUSION CRITERIA

We will include male and female adult patients (aged 18-99 years) with a diagnosis of lupus, regardless of active neurological disease.

4.3 EXCLUSION CRITERIA

We will exclude any lupus patients with life-threatening co-morbidities such as cancer (but if 'cured' or in long term remission then patients may be included depending on treatment and part of body affected). We will avoid scanning during concurrent acute illness like infection. Those with prior significant head injury or known neurological disease such as multiple sclerosis will be excluded. Patients with contra-indications to MR examination will be excluded. Those outwith the 18-99 years age range will be excluded.

5 PARTICIPANT SELECTION AND ENROLMENT

5.1 IDENTIFYING PARTICIPANTS / SCREENING FOR ELIGIBILITY / FIRST CONTACT

Potential participants will be identified by (a) the main Clinical Collaborator Dr Nicole Amft, or (b) NHS Lothian Consultant Rheumatologists responsible for the care of the patient.

Returning patients who are already known to the consultant may be sent information about the study prior to their next clinic visit so that they are aware in advance of their visit. New patients will be informed about the study at the clinic visit by the consultant and provided with the study information by the consultant or clinic nurse. NHS Lothian rheumatology nurses may assist with the practical aspects of this.

Identification will involve screening forthcoming NHS Lothian clinic lists for lupus patients, returning or new. Screening will be done by the NHS Lothian rheumatology service care team and not the study research team. Medical notes may be used to confirm a diagnosis of lupus (being the primary inclusion criteria). Personal data used will include name, age (to exclude non-adults) and address (to write to the potential participant). Information about the study will be displayed in the clinic (leaflets and notice boards).

For clarity, we will not use the Scottish Lupus Exchange database to identify potential participants. However, once a recruit has consented to participate in our study, we will check to see if they are also already participating in the SLEx project, and if so, will request data to be extracted from that database where it is of use to our project.

5.2 CONSENTING PROCESS

This step will occur after first approach has been undertaken by the Consultant Rheumatologist. The Principal Investigator will be informed about returning or new potentially interested patients by the Rheumatology service staff and will either meet returning patients to confirm their interest at the clinic, obtain consent and arrange study investigations, or follow up with the new patients after their clinic visit by phone or post to see if they are interested, obtain consent and arrange the appointments. Potential participants will have been given the Patient Information Sheet a minimum of 24 hours before consent is sought. Potential participants will be encouraged to ask questions. Consent will take place at the Western General Hospital. See also Section 8.2.1.

6 DATA COLLECTION

6.1 LOCATION

All research activity involving participants will take place at the Western General Hospital.

6.2 TIME

Data will be collected at one time point only, ie, this is a cross-sectional design. All investigations will be done in a study window of about 2 weeks, to avoid a long time lag between different measures.

6.3 BLOOD PROCESSING

A research nurse from the Wellcome Trust Clinical Research Facility (WTCRF) will perform venepuncture and collect samples for clinical and biomarker analysis. The blood will be spun, placed into aliquots and frozen at the WTCRF until batch analysed either once all 50 participants have had MR brain scanning.

6.4 BLOOD ANALYSIS

The blood will be batch analysed within NHS Lothian accredited haematology and biochemistry laboratories.

6.5 DEMOGRAPHICS AND BACKGROUND DATA

Stewart Wiseman will be the main data collector or co-ordinator. We will use medical notes and a Case Record Form designed for the study. The Study Team will not access medical notes until after consent is obtained.

6.6 MENTAL STATUS

Stewart Wiseman or the radiographers at the BRIC will assess anxiety, depression, cognition and childhood IQ using validated instruments used in previous studies of mild stroke at our Centre. The study participant will self-administer the Fatigue Severity Scale at the same session.

6.7 SCAN PROTOCOL

Stewart Wiseman or the radiographers at the BRIC will scan the participants. We will use a scan protocol[37] developed at our Centre for a large study on ageing, modified to exclude the 2 magnetisation transfer sequences which are not need for this study. Scanning will take 45-50 mins per subject. The MR imaging sequences are:

Scan sequence	Rationale
DTI	For tractography analysis
FSPGR 2 & 12	For tractography analysis
T2 loc	Mid-line localiser to set up other scans
T2	Assessment of PVS, lacunes, infarcts
GRE	Assessment of microbleeds / iron deposits; for other image processing
FLAIR	Aseeesment of WMH, infarcts; for other image proceesing
T1 volume	To produce T1-weighted maps for image analysis; tissue segmentation

6.8 LIST OF INSTRUMENTS

The following table itemises the validated tools we will use for assessment of mental status and lupus disease activity.

Tools for mental status
Fatigue Severity Scale (FSS)
Hospital Anxiety and Depression Scale (HADS)
Montreal Cognitive Assessment (MoCA)
Addenbrookes Cognitive Examination-Revised (ACE-R)
Mini Mental State Examination (MMSE)
National Adult Reading Test (NART)
Tools for lupus assessment
British Isles Lupus Assessment Group Index (BILAG)
SLE Disease Activity Index (SLEDAI)
Systemic Lupus International Collaborating Clinics Damage Index (SLICC)

7 STATISTICS AND DATA ANALYSIS

7.1 SAMPLE SIZE CALCULATION

This is a pilot study. There are no directly similar previous studies and the two studies that we found that used advanced MR imaging were small (n=34[27] and 8[28]). We plan to recruit 50 lupus patients.

7.2 PROPOSED ANALYSES

1. We will
   (a) visually assess (using validated rating scales) and;
   (b) take quantitative measurements (using semi-automated techniques)

from the structural MR imaging to assess cerebral SVD (ie, white matter hyperintensities, enlarged perivascular spaces, lacunes, acute small subcortical infarcts and atrophy).

2. We will use computational techniques to assess sub-visible damage to the brain's white matter.

3. We will use validated tools to assess cognition, depression and fatigue.

4. We will analyse blood for various markers of coagulation, fibrinolysis, inflammation and endothelial dysfunction as well as lupus disease activity.

Statistical analysis of primary outcome measures:

We will use descriptive statistics to answer our primary outcome of "do lupus patients show MR imaging evidence of cerebral SVD". We will use linear regression (from which we will get a correlation coefficient) to assess (a) association of brain imaging features with measures of fatigue, depression and cognitive impairment and (b) association of brain imaging features with measures of inflammation, lupus disease activity and accumulated rheumatic damage. We will use multiple parameters in the model to control for known vascular risk factors.

Statistical analysis of secondary outcome measures:

We will use either independent t-tests or Mann-Whitney U tests (depending on the shape of the data) to assess for differences between the lupus group and (a) historical controls with mild stroke in terms of various measureable brain imaging features and (b) historical healthy controls in terms of measures of white matter tract integrity.

8 GOOD CLINICAL PRACTICE

8.1 ETHICAL CONDUCT

The study will be conducted in accordance with the principles of the International Conference on Harmonisation Tripartite Guideline for Good Clinical Practice (ICH GCP).

A favourable ethical opinion will be obtained from the appropriate REC and local R&D approval will be obtained prior to commencement of the study.

8.2 INVESTIGATOR RESPONSIBILITIES

The Chief Investigator will be responsible for the overall conduct of the study at the site and compliance with the protocol and any protocol amendments. In accordance with the principles of ICH GCP, responsibilities may be delegated to an appropriate member of study site staff.

8.2.1 Informed Consent

See also Section 5.2 above. The Principal Investigator will be responsible for ensuring informed consent is obtained before any protocol specific procedures are carried out. The decision of a participant to participate in clinical research is voluntary and should be based on a clear understanding of what is involved.

Only adults, with the capacity to understand the research, will be invited to participate. If there is any doubt as to the individuals' ability to either understand the information provided, the individual will not participate in the study. Participants will be able to withdraw from the

study at any time without giving us a reason – we will make this clear to potential recruits in advance of participation and on the Patient Information Sheet.

A copy of the signed Consent Form will be: (a) given to the participant and (b) kept in the Study Site File.

8.2.2 Data Recording

The Principal Investigator will be responsible for the quality of the data recorded in the Case Record Form (CRF).

8.2.3 GCP Training

All study staff will hold evidence of appropriate GCP training.

8.2.4 Confidentiality

All patient brain scans (when sent for image analysis), cognitive assessments and blood results will be anonymised, although the link will not be fully broken as the patient's name will be on the Consent Form and a process of re-identification would be possible. Re-identification is necessary so that we may communicate with the participant and their healthcare providers, if necessary. Brain scans that go into the patient's medical records will not be anonymised (these will be stored on NHS computers just as any other medical scan data).

All patient identifiable data will be stored in paper format in a locked filing cabinet in a secure access room with a keycode and used only for necessary contact with the patient/ GP/ healthcare professionals.

Any dissemination of information following the study will not contain any patient identifiable information.

The Principal Investigator and study staff involved with this study will not disclose or use for any purpose other than performance of the study, any data, record, or other unpublished, confidential information disclosed to those individuals for the purpose of the study. Prior written agreement from the sponsor or its designee must be obtained for the disclosure of any said confidential information to other parties.

Anonymised study data (no personal identifiable data) will be available for use in future relevant research.

8.2.5 Data Protection

All Investigators and study site staff involved with this study will comply with the requirements of the Data Protection Act 1998 with regard to the collection, storage, processing and disclosure of personal information and will uphold the Act's core principles. Access to collated participant data will be restricted to those clinicians treating the participants.

Computers used to collate the data will have limited access measures via user names and passwords.

Published results will not contain any personal data that could allow identification of individual participants.

9 STUDY CONDUCT RESPONSIBILITIES

9.1 PROTOCOL AMENDMENTS

Any changes in research activity, except those necessary to remove an apparent, immediate hazard to the participant in the case of an urgent safety measure, must be reviewed and approved by the Chief Investigator.

Amendments to the protocol must be submitted in writing to the appropriate REC, Regulatory Authority and local R&D for approval prior to participants being enrolled into an amended protocol.

9.2 PROTOCOL VIOLATIONS AND DEVIATIONS

Investigators will not plan any deviation from the protocol except where necessary to eliminate an immediate hazard to participants.

In the event that an Investigator deviates from the protocol, the nature of and reasons for the deviation will be recorded in the CRF. Protocol deviations and violations will be reported to the Sponsor as per ACCORD SOP CR0010. If a subsequent protocol amendment is required, this should be submitted to the Sponsor for review and then to the REC and local R&D for approval if appropriate.

9.3 SERIOUS BREACH REQUIREMENTS

A serious breach is a breach which is likely to effect to a significant degree:

a) the safety or physical or mental integrity of the participants of the trial; or

b) the scientific value of the study.

If a potential serious breach is identified by the Chief investigator, Principal Investigator or delegates, the co-sponsors (accord.seriousbreach@ed.ac.uk) must be notified within 24 hours. It is the responsibility of the co-sponsors to assess the impact of the breach on the scientific value of the trial, to determine whether the incident constitutes a serious breach and take the appropriate action.

Not every violation from the protocol needs to be reported to the regulatory authority as a serious breach. If the sponsor(s) deem the incident to be a violation that does not constitute a serious breach from the protocol when identified, corrective and preventative actions will be taken where appropriate and they will be recorded in file notes, held within the Study Site File.

9.4 STUDY RECORD RETENTION

All study documentation will be kept for a minimum of 5 years from the protocol defined end of study point. When the minimum retention period has elapsed, study documentation will not be destroyed without permission from the sponsor.

9.5 END OF STUDY

The end of study is defined as the last participant's last visit. Analysis of the data and reporting of study results will persist for several months beyond this defined end date.

The Investigators and/or the trial steering committee and/or the co-sponsor(s) have the right at any time to terminate the study for clinical or administrative reasons.

The end of the study will be reported to the REC and Regulatory Authority within 90 days, or 15 days if the study is terminated prematurely. The Investigators will inform participants of the premature study closure and ensure that the appropriate follow up is arranged for all participants involved.

A summary report of the study will be provided to the REC and Regulatory Authority within 1 year of the end of the study.

9.6 INSURANCE AND INDEMNITY

The co-sponsors are responsible for ensuring proper provision has been made for insurance or indemnity to cover their liability and the liability of the Chief Investigator and staff.

The following arrangements are in place to fulfil the co-sponsors' responsibilities:

- The Protocol has been designed by the Principal and Chief Investigators and researchers employed by the University and collaborators. The University has insurance in place (which includes no-fault compensation) for negligent harm caused by poor protocol design by the Chief Investigator and researchers employed by the University.
- Sites participating in the study will be liable for clinical negligence and other negligent harm to individuals taking part in the study and covered by the duty of care owed to them by the sites concerned. The co-sponsors require individual sites participating in the study to arrange for their own insurance or indemnity in respect of these liabilities.
- Sites which are part of the United Kingdom's Nation Health Service will have the benefit of NHS Indemnity.

10 REPORTING, PUBLICATIONS AND NOTIFICATION OF RESULTS

10.1 AUTHORSHIP POLICY

Ownership of the data arising from this study resides with the study team. On completion of the study, the study data will be analysed and tabulated, and a clinical study report and papers for submission to peer-reviewed journals will be prepared in accordance with ICH guidelines.

10.2 PUBLICATION

The clinical study report will be used for publication and presentation at scientific meetings. Investigators have the right to publish orally or in writing the results of the study.

Summaries of results will also be made available to Investigators for dissemination within their clinics (where appropriate and according to their discretion).

11 APPENDICIES

The following separate electronic files form an integral part of this Study Protocol.

Type	Document description	File name
PowerPoint	Data Collection Diagram	**POSTCARD_CRF_DataCollectionDiagram_v1** (dated 26/11/13)
Microsoft Word	Letter of Invitation to prospective participants – sent in advance	**POSTCARD_LoI_sent_in_advance_v1** (dated 26/10/13)
Microsoft Word	Letter of Invitation to prospective participants – given at clinic visit to take away and consider	**POSTCARD_LoI_given_at_clinic_v1** (dated 18/11/13)
Microsoft Word	Letter to participants GP advising of participation	**POSTCARD_GP_Let_TakePart_v1** (dated 18/11/13)
Microsoft Word	Letter to NHS Lothian Consultant Rheumatologists	**POSTCARD_Let_to_Rheumatologists_v1** (dated 21/11/13)
Microsoft Word	Patient Information Sheet	**POSTCARD_PatientInfoLeaflet_v1** (dated 6/8/13)
Microsoft Word	Consent Form	**POSTCARD_PatientConsentForm_v1** (dated 17/11/13)
PowerPoint	Flyer	**POSTCARD_Flyer_v1** (dated 2/12/13)
Microsoft Word	Fatigue Severity Scale (FSS)	**POSTCARD_FSS_v1** (dated 14/11/13)
PDF	Hospital Anxiety and Depression Scale (HADS)	**HADS** (dated 14/11/13).
PDF	Montreal Cognitive Assessment (MoCA)	**MoCA-Test-English_7_3 June_13** (dated 14/11/13)
PDF	Addenbrookes Cognitive Examination-Revised (ACE-R)	
PDF	Mini Mental State Examination (MMSE)	
	National Adult Reading Test (NART)	
PDF	British Isles Lupus Assessment Group Index (BILAG)	**BILAG** (dated 14/11/13)
PDF	SLE Disease Activity Index (SLEDAI)	**SLEDAI** (dated 19/2/13)
PDF	Systemic Lupus International Collaborating Clinics Damage Index (SLICC)	**SLICC** (dated 19/2/13)
Microsoft Word	Scan sequences	**POSTCARD_SCAN_PARAM** (dated 17/5/11)

12 REFERENCES

1. Bessant R, Hingorani A, Patel L, MacGregor A, Isenberg DA and Rahman A. Risk of coronary heart disease and stroke in a large British cohort of patients with systemic lupus erythematosus. *Rheumatology*. 2004;43(7):924–9.

2. Futrell N and Millikan C. Frequency, etiology, and prevention of stroke in patients with systemic lupus erythematosus. *Stroke*. 1989;20(5):583–591.

3. Kitagawa Y, Gotoh F, Koto A and Okayasu H. Stroke in systemic lupus erythematosus [published erratum appears in Stroke 1991 Mar;22(3):417]. *Stroke*. 1990;21(11):1533–1539.

4. Krishnan E. Stroke subtypes among young patients with systemic lupus erythematosus. *The American Journal of Medicine*. 2005;118(12):1415.

5. Mok CC, Ho LY and To CH. Annual incidence and standardized incidence ratio of cerebrovascular accidents in patients with systemic lupus erythematosus. *Scandinavian Journal of Rheumatology*. 2009;38(5):362–8.

6. Urowitz MB, Gladman D, Ibañez D, Bae SC, Sanchez-Guerrero J, Gordon C, Clarke A, Bernatsky S, Fortin PR, Hanly JG, Wallace DJ, Isenberg D, Rahman A, Alarcón GS, Merrill JT, et al. Atherosclerotic vascular events in a multinational inception cohort of systemic lupus erythematosus. *Arthritis Care & Research*. 2010;62(6):881–7.

7. Chiu C-C, Huang C-C, Chan W-L, Chung C-M, Huang P-H, Lin S-J, Chen J-W, Leu H-B. Increased Risk of Ischemic Stroke in Patients with Systemic Lupus Erythematosus: A Nationwide Population-based Study. *Internal Medicine*. 2012;51(1):17–21.

8. Joseph FG, Scolding NJ. Neurolupus. *Practical Neurology*. 2010;10(1):4–15.

9. Steup-Beekman GM, Zirkzee EJM, Cohen D, Gahrmann BMA, Emmer BJ, Steens SCA, Bollen ELEM, van Buchem MA, Huizinga TWJ. Neuropsychiatric manifestations in patients with systemic lupus erythematosus: epidemiology and radiology pointing to an immune-mediated cause. *Annals of the Rheumatic Diseases*. 2013;72 Suppl 2(January 1989):ii76–9.

10. Hanly et al. Prospective analysis of neuropsychiatric events in an international disease inception cohort of SLE patients. *Ann Rheum Dis*. 2011;69(3):529–535.

11. Bosma GPT, Middelkoop HAM, Rood MJ, Bollen ELEM, Huizinga TWJ, van Buchem MA. Association of global brain damage and clinical functioning in neuropsychiatric systemic lupus erythematosus. *Arthritis and Rheumatism*. 2002;46(10):2665–72.

12. Cohen D, et al. Nationwide autopsy study links complement to thrombo-ischemia in neurolupus. University of Leiden. *Submitted*. 2013.

13. Bailey EL, Smith C, Sudlow CLM, Wardlaw JM. Pathology of lacunar ischaemic stroke in humans - A systematic review. *Brain Pathology*. 2012.

14. Gouw AA, Seewann A, van der Flier WM, Barkhof F, Rozemuller AM, Scheltens P, Geurts JJG. Heterogeneity of small vessel disease: a systematic review of MRI and histopathology correlations. *Journal of Neurology, Neurosurgery, and Psychiatry*. 2011;82(2):126–35.

15. Haley AP, Ph D, Hoth KF, Gunstad J, Paul RH, Jefferson AL, Tate DF, Ono M, Jerskey BA, Poppas A, Sweet LH, Cohen RA. Subjective Cognitive Complaints Relate to White Matter Hyperintensities and Future Cognitive Decline in Patients with Cardiovascular Disease. *Am J Geriatr Psychiatry*. 2010;17(11):1–17.

16. Saini M, Ikram K, Hilal S, Qiu A, Venketasubramanian N, Chen C. Silent stroke: not listened to rather than silent. *Stroke*. 2012;43(11):3102–4.

17. Wardlaw JM, Doubal FN, Valdes-Hernandez M, Wang X, Chappell FM, Shuler K, Armitage PA, Carpenter TC, Dennis MS. Blood-brain barrier permeability and long-term clinical and imaging outcomes in cerebral small vessel disease. *Stroke*. 2013;44(2):525–7.

18. Wardlaw JM, Smith C, Dichgans M. Mechanisms of sporadic cerebral small vessel disease: insights from neuroimaging. *Lancet Neurology*. 2013;12(5):483–497.

19. Debette S, Markus HS. The clinical importance of white matter hyperintensities on brain magnetic resonance imaging: systematic review and meta-analysis. *BMJ*. 2010;341(jul26 1):c3666–c3666.

20. Chinn RJ, Wilkinson ID, Hall-Craggs M a, Paley MN, Shortall E, Carter S, Kendall BE, Isenberg D a, Newman SP, Harrison MJ. Magnetic resonance imaging of the brain and cerebral proton spectroscopy in patients with systemic lupus erythematosus. *Arthritis and Rheumatism*. 1997;40(1):36–46.

21. Ainiala H, Dastidar P, Loukkola J, Lehtimäki T, Korpela M, Peltola J, Hietaharju a. Cerebral MRI abnormalities and their association with neuropsychiatric manifestations in SLE: a population-based study. *Scandinavian Journal of Rheumatology*. 2005;34(5):376–82.

22. Stimmler MM, Coletti PM, Quismorio FP. Magnetic resonance imaging of the brain in neuropsychiatric systemic lupus erythematosus. *Seminars in Arthritis and Rheumatism*. 1993;22(5):335–49.

23. Sanna G, Piga M, Terryberry JW, Peltz MT, Giagheddu S, Satta L, Ahmed a., Cauli a., Montaldo C, Passiu G, Peter JB, Shoenfeld Y, Mathieu A. Central nervous system involvement in systemic lupus erythematosus: cerebral imaging and serological profile in patients with and without overt neuropsychiatric manifestations. *Lupus*. 2000;9(8):573–583.

24. Oku K, Atsumi T, Furukawa S, Horita T, Sakai Y, Jodo S, Amasaki Y, Ichikawa K, Amengual O, Koike T. Cerebral imaging by magnetic resonance imaging and single photon emission computed tomography in systemic lupus erythematosus with central nervous system involvement. *Rheumatology*. 2003;42(6):773–7.

25. Abreu MR, Jakosky A, Folgerini M, Brenol JCT, Xavier RM, Kapczinsky F. Neuropsychiatric systemic lupus erythematosus: correlation of brain MR imaging, CT, and SPECT. *Clinical Imaging*. 2005;29(3):215–21.

26. Katsumata Y, Harigai M, Kawaguchi Y, Fukasawa C, Soejima M, Kanno T, Nishimura K, Yamada T, Yamanaka H, Hara M. Diagnostic reliability of magnetic resonance imaging for

central nervous system syndromes in systemic lupus erythematosus: a prospective cohort study. *BMC musculoskeletal disorders*. 2010;11:13.

27. Zhang L, Harrison M, Heier L a, Zimmerman RD, Ravdin L, Lockshin M, Uluğ AM. Diffusion changes in patients with systemic lupus erythematosus. *Magnetic Resonance Imaging*. 2007;25(3):399–405.

28. Hughes M, Sundgren PC, Fan X, Foerster B, Nan B, Welsh RC, Williamson J a, Attwood J, Maly P V, Chenevert TL, McCune W, Gebarski S. Diffusion tensor imaging in patients with acute onset of neuropsychiatric systemic lupus erythematosus: a prospective study of apparent diffusion coefficient, fractional anisotropy values, and eigenvalues in different regions of the brain. *Acta radiologica*. 2007;48(2):213–22.

29. Sibbitt WL, Brooks WM, Kornfeld M, Hart BL, Bankhurst AD, Roldan CA. Magnetic resonance imaging and brain histopathology in neuropsychiatric systemic lupus erythematosus. *Seminars in Arthritis and Rheumatism*. 2010;40(1):32–52.

30. Petri M, Roubenoff R, Dallal GE, Nadeau MR, Selhub J, Rosenberg IH. Plasma homocysteine as a risk factor for atherothrombotic events in systemic lupus erythematosus. *Lancet*. 1996;348(9035):1120–4.

31. Wiseman S, Marlborough F, Doubal F, Webb DJ, Wardlaw J. Blood markers of coagulation, fibrinolysis, endothelial dysfunction and inflammation in lacunar stroke versus non-lacunar stroke and non-stroke: systematic review and meta-analysis. *Cerebrovascular Dis*. 2014. In production.

32. Bazner H, Blahak C, Pantoni L, Inzitari D, Hennerici M, on behalf of the LADIS study group. Progressive motor compromise is closely related to disability and death in age-related white matter changes 3-year results of the LADIS study. *Cerebrovascular Dis*. 2008.

33. Mead G, Lewis SC, Wardlaw J, Dennis M, Warlow C. Severe ipsilateral carotid stenosis and middle cerebral artery disease in lacunar ischaemic stroke: innocent bystanders? *J Neurol*. 2002.

34. Benavente OR, Hart RG, McClure L a, Szychowski JM, Coffey CS, Pearce L a. Effects of clopidogrel added to aspirin in patients with recent lacunar stroke. *The New England Journal of Medicine*. 2012;367(9):817–25.

35. Palacio S, Hart RG, Pearce L a, Benavente OR. Effect of addition of clopidogrel to aspirin on mortality: systematic review of randomized trials. *Stroke*. 2012;43(8):2157–62.

36. Valdés Hernández MDC, Booth T, Murray C, Gow AJ, Penke L, Morris Z, Maniega SM, Royle NA, Aribisala BS, Bastin ME, Starr JM, Deary IJ, Wardlaw JM. Brain white matter damage in aging and cognitive ability in youth and older age. *Neurobiology of Aging*. 2013;34(12):2740–7.

37. Wardlaw JM, Bastin ME, Valdés Hernández MC, Maniega SM, Royle NA, Morris Z, Clayden JD, Sandeman EM, Eadie E, Murray C, Starr JM, Deary IJ. Brain aging, cognition in youth and old age and vascular disease in the Lothian Birth Cohort 1936: rationale, design and methodology of the imaging protocol. *International Journal of Stroke*. 2011;6(6):547–59.

Appendix G: Patient information sheet

Neuroimaging Sciences
University of Edinburgh
Department of Clinical Neurosciences
Western General Hospital
Crewe Road
Edinburgh EH4 2XU
www.bric.ed.ac.uk

Principal Investigator: Stewart Wiseman swiseman@staffmail.ed.ac.uk Tel: 0131 537 2660 or 0131 537 1985
Chief Investigator: Professor Joanna Wardlaw

Pilot MRI scanning study of the brains of patients with lupus

Patient Information Sheet

You are being invited to take part in a study into how lupus affects the brain. This Patient Information Sheet is designed to give you clear information about the study, and your involvement if you decide to take part. Please take time to read it, and discuss it with others if you wish. Ask us if there is anything that is not clear, or if you need more information. Take time to decide whether or not you wish to take part. Your current and future treatment will not be affected whatever you decide.

Why am I being invited, and do I need to take part?

You are being invited to take part because you have been diagnosed with lupus (also known as systemic lupus erythematosus or SLE). You do not need to take part. If you are interested in the study, taking part would be voluntary.

What is the study about?

People with lupus often have neurological (brain) symptoms. These can be mild or severe and can vary from patient to patient. Examples include problems with memory or mood. More serious problems might include seizures or strokes. Even though we have known for a long time that the brain is affected in this disease, we don't know that much about it. We want to scan the brains of people with lupus (whether they have brain symptoms or not). We will look at the scans and take some measurements from them. We also want to do some other tests (see "If I take part, what do I have to do"). We are interested in the small blood vessels in the brain and also the brain's wiring (doctors call this the "white matter"). Things could be happening to these parts of the brain that explain some of the brain issues.

If I take part, what do I have to do?

We want to scan your brain with a magnetic resonance scanner (usually just called MRI), do an assessment of your anxiety, depression, cognition and fatigue and take a blood sample (the amount would be about 5 or 5.5 teaspoons) and a blood pressure reading. The MRI scan would last about 50 mins. The other tests would be about a further 45 mins. So, you would need to set aside up to 2 hours. All tests are performed at the Western General Hospital in Edinburgh.

Are there any risks?

Your care will not be affected. You will not be given any experimental drugs. Sometimes, people get claustrophobic in the MRI scanner - be assured that we are very well aware of this and geared up to deal with it. We will explain everything on the day you are being scanned. You can also get information before hand (by phoning us or visiting our web site) and we can arrange for you to see the scanner beforehand if that helps. Please note that people with a cardiac pacemaker, aneurism clips or cochlear implant are not allowed to go in an MRI scanner. We will go over this and all the other safety issues with you before you are scanned so you are comfortable and informed about the procedure. The blood sample will involve a small needle in the arm, similar to what normally happens at your GP or the rheumatology clinic.

Who is running the study and where is it being held?

The study is being run by the University of Edinburgh and NHS Lothian. We will do the study at the Western General Hospital, Edinburgh. Our website can be found here: www.bric.ed.ac.uk.

Will I get paid?

No. However, we can cover the cost of you getting to and from the hospital if you decide to take part.

Your expectations about the scan results

Your individual scan results are **not** meant to diagnose you, even if you have had neurological problems as part of your lupus. Your individual involvement is very gratefully appreciated by us, but it is the combined results from scanning many people that gives us most information. However, your scan and a radiological report will be put into your NHS records so that the doctors looking after you can see it.

What is an MRI?

MRI is a type of brain scanner. It is very common in hospitals but we also use it for research. An MRI scan is very safe and does not involve radiation. We have lots of expertise in brain scanning, and the Principal and Chief Investigators of the study, Stewart Wiseman and Joanna Wardlaw, are a fully qualified MRI radiographer and neuroradiologist, respectively. The scanner is noisy and so we will give you ear protection (little foam pads that go in the ear and pads on the outside). You will need to lie on your back for about 50 minutes; your head will be in the middle of the scanner. The scanner is open at both ends so you will not be boxed in. However some people have become claustrophobic in the scanner. We are used to dealing with this, so don't worry. Feel free to speak to any of the radiographers before you come for your scan. Please note that people with a cardiac pacemaker, aneurism clips or cochlear implant are not allowed to go in an MRI scanner. We will go over this and all the other safety issues with you beforehand, so again don't worry. We have a list of frequently asked questions (FAQs) about MRI and taking part in brain research here: http://www.bric.ed.ac.uk/studySub/subjectquestions.html. If you do not have access to the internet, please contact us and we will send you a copy of the FAQs in the post.

What if you find something unusual on my brain scan or other tests?

Your scan will be looked at by a neuroradiologist (a 'consultant level' senior doctor that specialises in brain scans) and a report sent to your rheumatologist. A report of the scan will form part of your medical records. Also, if we find anything untoward (we call this "***an incidental finding***") we will tell your rheumatologist who will pass on relevant information to yourself and your GP. Incidental findings are found in about 3% of people who have a brain scan for research, and most are not worth worrying about. The rate of serious incidental findings (eg, a brain tumour) is much less than 1%. The rheumatologist and/or GP will then be able to advise on and arrange the best course of action, in discussion with yourself. We will also tell your rheumatologist if any of the blood tests or tests to assess anxiety, depression, fatigue and cognition are abnormal. If we do find something unusual on your brain scan you would be treated on the NHS. In cases of brain tumours this could involve having surgery. The scans, and the expertise and medical opinion of our neuroradiologist, would be available to the NHS doctors. The scans we will do as part of the study are likely to be much more detailed than an NHS scan which would be beneficial in the unlikely event that we did find something. It is important to note that if we discover an abnormality (such as a brain tumour) this could have implications for your future applications for insurance – insurance companies (life, travel) want to know these things as it affects how they assess an application.

Will my GP be told about my participation?

Yes, we will write to your GP to tell them you are taking part. Your GP will also receive information about the scan and other tests from your rheumatologist, if they deem this appropriate.

Scottish Lupus Exchange Database

You might have heard about the new Scottish Lupus Exchange (SLEx) Database project or even be participating in it. We are working with the people running the SLEx database project. By collecting information from patients with lupus, the SLEx project hopes to better understand the number of patients with lupus in Scotland, when and how they were diagnosed and what complications or problems they experience because of this disease. They are also studying the effectiveness of treatments.

Will I be contacted by anyone else, or will the study team contact me again in the future?

If you are already in the SLEx project, we will ask your permission to share your brain scan and other results with them. We will not pass on your details to anyone else. We will also ask your permission for our study team to contact you in the future. You do not need to take part in any future studies. Future studies would supply you with a new Patient Information Sheet.

Will my data be kept safe and confidential?

Any data we obtain from you or your medical records will be kept strictly confidential. Information will be stored securely in accordance with the Data Protection Act 1998. If you agree to take part, we will assign a code to you. When your data is being analysed it will be by code. Your name and other personal data will be kept separately and only knowable by the study team. However, we may be required by regulatory authorities (the people that ensure research studies are being conducted properly) to inspect your records – they do this for your benefit by checking we are running the study properly.

Consenting

You will have been given this Patient Information Sheet by your rheumatologist or the rheumatology nurses. In order to join the study you will be asked to sign a Consent Form. Before you sign, you should understand the study and what is expected of you by participating. We will not ask you to consent until you have had this Patient Information Sheet for 24 hours – so that you have time to consider the study. Take longer if you need to, and ask questions if there is anything unclear. The Consent Form will be given to you by the main researcher, Stewart Wiseman, who will go over it with you once you are ready. If you lost capacity during the study (eg, if you became very unwell and were no longer able to make decisions for yourself) we would keep the data collected from you.

If I join the study, can I leave after it has started?

Yes. You can stop taking part at any stage. You do not need to give us a reason. Data collected on you up to the time you leave the study will either be destroyed or kept (this decision on whether the data is kept or destroyed is yours). No new data will be collected on you after you leave.

Will joining this study affect my medical care?

No. You will receive the same medical care whether you are in the study or not.

Can I find out the results of the study?

Yes. We will write to you at the end of the study. We also hope to publish the results in scientific journals.

How long will the study last, and what happens to my data at the end?

The study will run throughout 2014, possibly into early 2015. We are required by law to keep a record of everything that happened and the data that was collected for a minimum of 5 years. Please note that it is common for new research questions to come up after a study is finished and we would like to be able to use your data, anonymously, to answer future relevant research questions. Your data would be coded to maintain your anonymity, ie, your name or other personal details will not be used.

Who is funding this study?

The study is being funded by Edinburgh University and the charity Lupus UK.

How can I travel to the hospital?

Parking is limited at the hospital, and the main car park is at the other end of the hospital from the Department of Clinical Neurosciences (DCN) where the MRI scanner is located. DCN is located at the Telford Road end of the hospital and has a smaller car park (car park 8) which you are welcome to try but please note that getting a space is often difficult. If coming by car we recommend you give yourself extra time before the scanning appointment time. The hospital is well served by buses. We could arrange for a taxi to bring you from home to the hospital and back, which we will pay for. Please let us know if you need a taxi and we will book this for you.

Can I get independent advice about this study?

Dr William Whiteley is a neurologist at the Western General Hospital. He has nothing to do with this study. He has agreed to discuss the study with any lupus patient considering joining the study before they consent to take part, if they wish, or during the study if questions arise. He can be contacted on 0131 537 1089.

Where can I get more information?

For more information about the study please contact:

Stewart Wiseman
0131 537 1985 or 0131 537 2660

To discuss any aspect of having an MRI scan, please speak to any of the radiographers:

0131 537 2660

Who has reviewed this study and who do I turn to if things go wrong

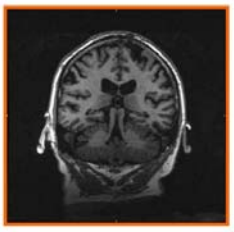
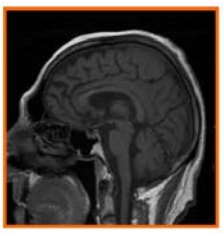
The study has been reviewed by South East Scotland Research Ethics Committee 1 under reference 14/SS/0003. They have raised no objections to the study. If you have any complaints about this study they should be addressed to the Principal Investigator in the first instance. If you do not get a satisfactory response you are free to contact the senior study team members:

Professor Joanna Wardlaw
Neuroimaging Sciences
University of Edinburgh
Western General Hospital
Crewe Road South, Edinburgh
0131 537 2664
Joanna.Wardlaw@ed.ac.uk

Professor Stuart Ralston
Molecular Medicine Centre
University of Edinburgh
Western General Hospital
Crewe Road South, Edinburgh
0131 651 1035
Stuart.Ralston@ed.ac.uk

You also have the right to complain to: NHS Lothian Complaints Team, Waverley Gate, 2 - 4 Waterloo Place, Edinburgh, EH1 3EG. Tel: 0131 536 3370 Email: complaints.team@nhslothian.scot.nhs.uk

~ Thank you for taking the time to read this leaflet and considering the study. ~

Appendix H: Patient consent form

Neuroimaging Sciences
University of Edinburgh
Department of Clinical Neurosciences
Western General Hospital
Crewe Road
Edinburgh EH4 2XU
www.bric.ed.ac.uk

Patient Consent Form

Patient Code: ☐ Patient Initials: ☐

1. I confirm I have read and understood the Patient Information Sheet "***Pilot MRI scanning study of the brains of patients with lupus***" version 3 (dated 8 April 2014).	Initials
2. I have had time to consider the study and have had all my questions about it answered.	Initials
3. I understand my participation is voluntary and that I am free to withdraw at any time, without giving a reason, and without it affecting my medical care.	Initials
4. I understand that the information collected about me will be held in confidence and that my name or other personal data will not appear in any publication.	Initials
5. I agree to you writing to my GP to tell him/her that I am in this study.	Initials
6. I agree to a copy of my scan report being sent to my Consultant Rheumatologist who will pass on any information of relevance, or that is incidental but worthy of medical note, to my GP.	Initials
7. I understand that a copy of the brain scan will go into my NHS records.	Initials
8. I give permission for the Principal Investigator and other responsible individuals in the study team to access my NHS medical records (for information relating to my lupus disease) as part of this study, and use information that is relevant.	Initials
9. I agree that the information collected for the study, including my brain scans, cognitive test results and blood sample, may be used in future in other relevant research and shared with the Scottish Lupus Exchange database project, as long as my personal data (such as my name) is anonymised.	Initials
10. I agree that the study team may continue to use my data for the purposes of the study, even if I lose capacity during the study.	Initials
11. I agree that the study team may keep my personal data (such as my name and address) for the purpose of contacting me in the future about new lupus research studies. There is no obligation on me to take part in future studies.	Initials
12. I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from the regulatory authorities and from the Sponsors (NHS Lothian and the University of Edinburgh) where it is relevant to my taking part in this research. I give permission for those individuals to have access to my records.	Initials
13. I agree to take part in this study.	Initials

Patient Name:_____ Signature:_____ Date:_____

Person taking consent:_____ Signature:_____ Date:_____

Original (x1) to be retained in site file. Copy (x1) to be retained by the participant.

Appendix I: Data collection form

Pilot MRI scanning study of the brains of patients with lupus (E131321) DATA COLLECTION FORM

Patient Code: LUP_ _ _ Patient Initials:

Neuroimaging Sciences
University of Edinburgh
DCN
Western General Hospital
Crewe Road
Edinburgh EH4 2XU
Tel: 0131 537 2660 or 0131 537 1985

Principal Investigator: Stewart Wiseman swiseman@staffmail.ed.ac.uk 07713 872 172
Chief Investigator: Professor Joanna Wardlaw

Radiographers' Checklist Consent Form (Stewart to do first)	**Tick**
Height and weight	
Scan	
Blood pressure reading	
This Case Record Form (**CRF**)	
Cognitive tests:	
(1) Hospital Anxiety and Depression Scale (**HADS**)	
(2) Montreal Cognitive Assessment (**MoCA**)	
(3) Addenbrooke's Cognitive Examination – Revised (**ACER**)	
(4) Mini Mental State (**MMSE**)	Derive score from Addenbrooke's
(5) Fatigue Severity Scale (**FSS**)	
(6) National Adult Reading Test (**NART**)	
Blood draw (WTCRF nurse)	
BILAG, SLEDAI, SLICC (from rheumatology / SLEx database)	Stewart to get

Today's date (dd-mmm-yyyy)		Completed by:	

		First reading	**Second reading**	**Third reading**
Blood Pressure Record Systolic and Diastolic: Example 120/80	First reading = before enter scan room. Second reading = on leaving scanner. Third = on leaving the department.			

Height	in cm	
Weight	in kg	
Handedness		

Patient Code: LUP_ _ _ Patient Initials:

Patient Data

***** DO NOT STORE PATIENT-IDENTIFIABLE DATA ON THIS PAGE (Page 2) ELECTRONICALLY *****

***** THIS PAPER FORM TO BE LOCKED AWAY *****

Patient Name:	
Patient Address:	
Patient telephone (home + mobile):	
CHI:	
GP:	
Consultant Rheumatologist:	

Trak film packet and/or visit labels:

Patient Code: LUP_ _ _ Patient Initials:

SLEx

Are you taking part in the new Scottish Lupus Exchange registry database? Ask participant. Stewart to verify with Caroline.	Code as: (1) Yes (2) No (99) Unknown	

Demographics

DoB (dd-mmm-yyyy)			
Sex Code as: (1) Female (2) Male		Age (yy)	
Ethnicity Lupus more prevalent in Afro-Caribbean populations so useful to capture ethnicity to check for confounding.	Code as: (1) Afro-Caribbean (2) Asian (3) Caucasian (white) (4) Oriental (5) Hispanic (6) Other (99) Unknown		

Education

What age did you leave secondary (high) school?		
What is the highest level of academic qualification obtained? Code as: (1) None (2) O grade / O level or equivalent (3) Scottish Higher / English A level (4) FE college, eg, HNC / HND	(5) Undergraduate degree (6) Masters / Post-graduate degree (7) PhD (99) Unknown	
Free text, eg, leaving certificate:		

Activity Level

This is needed to calculate the BMI score

How would you classify your daily activity levels? Activity not just exercise, can be as a result of an active job, caring for someone, etc	Code as: (1) Inactive (2) Low active (3) Moderate active (4) High active	

Patient Code: LUP_ _ _ Patient Initials:

Lupus history

Date of diagnosis Stewart will cross-ref with Trak, but useful to get the participant's recollection recorded here. THIS IS AN IMPORTANT QUESTION as we will be assessing brain features against length of time since diagnosis	Code as: If possible get a MMM and YYYY but as a minimum try and get YYYY. (99) Unknown	
Lupus medication name Code as: (0) I am not on any meds specifically for lupus (1) I am on something, not sure name (free text) Enter medication name ->>br>(99) Unknown		
Lupus medication name Code as: Use space for recording more than one (meds must be directly related to their lupus diagnosis)		

Some prompts to help with medication names:
(1) NSAID (Naproxen, Diclofenac)
(2) Anti-malarial (Hydroxychloroquine, Chloroquine)
(3) Immunosuppressants (Azathioprine, Methotrexate, Mycophenolate Mofetil, Cyclophosphamide)
(4) Corticosteriods (oral, topical, IV, rarely IM) – eg. Prednisolone (and variants)
(5) Biologicals (Rituximab, belilumab)

Have you been diagnosed with Neurolupus? Also called CNS Lupus Stewart to verify with Consultant / Trak.	Code as: (1) Yes (2) No (99) Unknown	
Date of diagnosis	Code as: If possible get a MMM and YYYY but as a minimum try and get YYYY. (99) Unknown	

Patient Code: LUP_ _ _ Patient Initials:

APS

Antiphospholipid syndrome (APS), also known as sticky blood or Hughes syndrome, is a clotting disorder, which can occur on its own or alongside lupus. Diagnosed by 2 antibody tests but can be negative in 20% of people with APS. Also need to assess for clots or miscarriage on 2 occasions at least 12 weeks apart. Source: Arthritis Research UK. Notes from Dr Makin: Warfarin is anti-coagulant therapy, not to be confused with anti-platelet therapy such as aspirin and clopidogrel. Warfarin is for those patients at higher risk of further embolic stroke due to AF

Have you been diagnosed with APS? Stewart to verify with Trak.	Code as: (1) Yes (2) No (99) Unknown	
Number of miscarriages? Females only	Code as: number given by participant 0 for none. (99) Unknown	
Are you on Warfarin (or equivalent eg, dabigatran or rivaroxaban)	Code as: (1) Yes (2) No (99) Unknown	

Patient Code: LUP_ _ _ Patient Initials:

Stroke history

Have you ever been diagnosed as having a TIA by a doctor? Prompt participant with "transient ischemic attack" and "mini stroke" – symptoms resolved in 24h	Code as: (1) Yes (2) No (99) Unknown	
Date	Get accurate a date as possible, but minimum YYYY Stewart to cross-reference with Trak (99) Unknown	
Have you ever been diagnosed as having a stroke by a doctor? Prompt with symptoms (slurred speech; drooping arm or face; problems with vision, etc….) usually rapid onset, symptoms persist >24h	Code as: (1) Yes (2) No (99) Unknown	
Date	Get accurate a date as possible, but minimum YYYY Stewart to cross-reference with Trak (99) Unknown	
Ask participant if they were started on medication as a result of stroke Record multiple responses. Will code in database as free text.	Prompt with examples: (1) Aspirin (2) Clopidogrel (3) Warfarin (4) BP lowering drugs (5) lipid lowering drugs (6) Yes but don't know name (99) Unknown	
Have you ever been diagnosed as having Atrial Fibrillation? An arrhythmia is a problem with the rate or rhythm of the heartbeat. During an arrhythmia, the heart can beat too fast, too slow, or with an irregular rhythm.	Code as: (1) Yes (2) No (99) Unknown	
Any other CNS involvement? Eg: Seizure, Organic brain syndrome		

Has either of your parents ever had a stroke? Free text notes:	Code as: (1) No (2) Father (3) Mother (4) Both parents (5) Grandfather (6) Grandmother (99) Unknown	
Has any of your siblings ever had a stroke (brother / sister)? Free text notes:	Code as: (1) Yes (2) No (3) I am an only child (99) Unknown	

Patient Code: LUP_ _ _ Patient Initials:

Vascular Risk Factors

Diabetes. Have you been diagnosed as diabetic?	Code as: (1) Yes (2) No (99) Unknown	
Type	Code as: (1) Type 1 (2) Type 2 (99) Unknown	

Smoking. Previously smoked means as a smoker (someone who would buy cigarettes) rather than 1 in the pub every so often	Code as: (1) Current Smoker (2) Previous smoker (3) Never smoked	
If you are a current smoker, how many per day on average?	Code as: number of cigarettes (or equivalent) (99) Unknown	
What age did you start?	Code as: age given (99) Unknown	

Alcohol. In a **typical** week (eg, not special events) how many times a week do you drink?	Code as: (1) Daily (2) Regularly (4 or 5 times in a week) (3) Moderately (2 or 3 times in a week) (4) Occasionally (1 day per week, or less) (5) I do not drink alcohol (99) Unknown	

Everybody's perception of "alcohol units" differs. Likewise alcohol strength and volume can differ, eg, a pint of beer can be 2% to 8% while a glass of wine can be 125ml or 250ml. Just need to be pragmatic and get what info we can:

1 Unit = 1 small glass of wine; 1 x 25ml gin or similar spirit; ½ pint normal strength lager

Alcohol. In a **typical** week (eg, not special events) how many units do you think?	Code as: (Number units estimated) (0) for non-drinkers (99) Unknown	

Patient Code: LUP_ _ _ Patient Initials:

Hypertension. Have you been diagnosed by a doctor as hypertensive (high blood pressure)? Hypertension defined as a reading over 140/90 mmHg, or on current medication for high BP.	Code as: (1) Yes (2) No (99) Unknown	
Are you on medication for high BP?	Code as: (1) Yes (2) No (99) Unknown	

High Cholesterol. Have you been diagnosed by a doctor with high cholesterol.	Code as: (1) Yes (2) No (99) Unknown	
Are you on medication for high cholesterol?	Code as: (1) Yes (2) No (99) Unknown	

Hormone Status Females only.

Are you post-menopause?	Code as: (1) Yes (2) No (99) Unknown	

Are you on oestrogen for bone protection?	Code as: (1) Yes (2) No (99) Unknown	

THANK THE PARTICIPANT.

NOW DO COGNITIVE ASSESSMENTS

Appendix J: Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K)

Neuroimaging Sciences
University of Edinburgh
DCN
Western General Hospital
Crewe Road
Edinburgh EH4 2XU
Tel: 0131 537 2660 or 0131 537 1985

Principal Investigator: Stewart Wiseman swiseman@staffmail.ed.ac.uk
Chief Investigator: Professor Joanna Wardlaw

SLEDAI

Patient Code: Patient Initials:

SYSTEMIC LUPUS ERYTHEMATOSUS DISEASE ACTIVITY INDEX
SELENA MODIFICATION

Physicians Global Assessment - please circle one of: **None Mild Med Severe**

Circle in SCORE column if descriptor is present at the time of visit or in the proceeding 10 days

SCORE	Descriptor	Definition
8	Seizure	Recent onset. Exclude metabolic, infectious or drug cause
8	Psychosis	Altered ability to function in normal activity due to severe disturbance in the perception of reality. Include hallucinations, incoherence, marked loose associations, impoverished thought content, marked illogical thinking, bizarre, disorganized, or catatonic behavior. Excluded uremia and drug causes.
8	Organic Brain Syndrome	Altered mental function with impaired orientation, memory or other intelligent function, with rapid onset fluctuating clinical features. Include clouding of consciousness with reduced capacity to focus, and inability to sustain attention to environment, plus at least two of the following: perceptual disturbance, incoherent speech, insomnia or daytime drowsiness, or increased or decreased psychomotor activity. Exclude metabolic, infectious or drug causes.
8	Visual Disturbance	Retinal changes of SLE. Include cytoid bodies, retinal hemorrhages, serious exodate or hemorrhages in the choroids, or optic neuritis. Exclude hypertension, infection, or drug causes.
8	Cranial Nerve Disorder	New onset of sensory or motor neuropathy involving cranial nerves.
8	Lupus Headache	Severe persistent headache: may be migrainous, but must be non-responsive to narcotic analgesia.
8	CVA	New onset of cerebrovascular accident(s). Exclude arteriosclerosis
8	Vasculitis	Ulceration, gangrene, tender finger nodules, periungual, infarction, splinter hemorrhages, or biopsy or angiogram proof of vasculitis

Cont…

SCORE	Descriptor	Definition
4	Arthritis	More than 2 joints with pain and signs of inflammation (i.e. tenderness, swelling, or effusion).
4	Myositis	Proximal muscle aching/weakness, associated with elevated creatine phosphokinase/adolase or electromyogram changes or a biopsy showing myositis.
4	Urinary Casts	Heme-granular or red blood cell casts
4	Hematuria	>5 red blood cells/high power field. Exclude stone, infection or other cause.
4	Proteinuria	>0.5 gm/24 hours. New onset or recent increase of more than 0.5 gm/24 hours.
4	Pyuria	>5 white blood cells/high power field. Exclude infection
2	New Rash	New onset or recurrence of inflammatory type rash.
2	Alopecia	New onset or recurrence of abnormal, patchy or diffuse loss of hair.
2	Mucosal Ulcers	New onset or recurrence of oral or nasal ulcerations
2	Pleurisy	Pleuritic chest pain with pleural rub or effusion, or pleural thickening.
2	Pericarditis	Pericardial pain with at least 1 of the following: rub, effusion, or electrocardiogram confirmation.
2	Low Complement	Decrease in CH50, C3, or C4 below the lower limit of normal for testing laboratory.
2	Increased DNA binding	>25% binding by Farr assay or above normal range for testing laboratory
1	Fever	>38°C. Exclude infectious cause
1	Thrombocytopenia	<100,000 platelets/mm3
1	Leukopenia	<3,000 White blood cell/mm3. Exclude drug causes.

_____ TOTAL SCORE (Sum of SCORES circled)

Appendix K: British Isles Lupus Assessment Group 2004 (BILAG)

BILAG (British Isles Lupus Assessment Group index)

Patient_____ Date_____

All features must be attributable to SLE and refer the last four weeks compared with the prior visit's disease activity.
Indicate and score which features are present: 0 = Not Present, 1 = Improving, 2 = Same, 3 = Worse, 4 = New or Recurrence.

General – MUST BE SLE RELATED		**Neurological - MUST BE SLE RELATED**	
1. Pyrexia (documented)	0 1 2 3 4	24. Deteriorating level of consciousness	0 1 2 3 4
2. Weight Loss – unintentional >5%	0 1 2 3 4	25. Acute psychosis, delirium, confusion	0 1 2 3 4
3. Lymphadenopathy/Splenomegaly	0 1 2 3 4	26. Seizures	0 1 2 3 4
4. Fatigue/Malaise/Lethargy	0 1 2 3 4	27. Stroke or stroke syndrome	0 1 2 3 4
5. Anorexia/nausea/vomiting	0 1 2 3 4	28. Aseptic Meningitis	0 1 2 3 4
Mucocutaneous - MUST BE SLE RELATED		29. Mononeuritis multiplex	0 1 2 3 4
6. Maculopapular eruption – severe, active, (bullous)	0 1 2 3 4	30. Ascending or transverse myelitis	0 1 2 3 4
7. Maculopapular eruption – mild	0 1 2 3 4	31. Peripheral or cranial neuropathy	0 1 2 3 4
8. Active discoid lesions – generalized / extensive	0 1 2 3 4	32. Disc swelling/cytoid bodies	0 1 2 3 4
9. Active discoid lesions – localized including lupus profundus	0 1 2 3 4	33. Chorea	0 1 2 3 4
10. Alopecia (severe, active)	0 1 2 3 4	34. Cerebellar ataxia	0 1 2 3 4
11. Alopecia (mild)	0 1 2 3 4	35. Headache severe, unremitting	0 1 2 3 4
12. Panniculitis (severe)	0 1 2 3 4	36. Organic depressive illness	0 1 2 3 4
13. Angioedema	0 1 2 3 4	37. Organic brain syndrome including	0 1 2 3 4
14. Extensive mucosal ulceration	0 1 2 3 4	Pseudotumor cerebri	0 1 2 3 4
15. Small mucosal ulcers	0 1 2 3 4	38. Episodic migranous headaches	0 1 2 3 4
16. Malar erythema	0 1 2 3 4	**Musculoskeletal - MUST BE SLE RELATED**	
17. Subcutaneous nodules	0 1 2 3 4	39. Definite myositis (Bohan & Peter)	0 1 2 3 4
18. Perniotic Skin Lesions	0 1 2 3 4	40. Severe Polyarthritis with loss of function	0 1 2 3 4
19. Periungal erythema	0 1 2 3 4	41. Arthritis	0 1 2 3 4
20. Swollen fingers	☐Yes ☐No	42. Tendonitis	0 1 2 3 4
21. Sclerodactyly	☐Yes ☐No	43. Mild chronic myositis	0 1 2 3 4
		44. Athralgia	0 1 2 3 4
22. Calcinosis	☐Yes ☐No	45. Myalgia	0 1 2 3 4
23. Telangiectasia	☐Yes ☐No	46. Tendon contractures and fixed deformity	☐Yes ☐No
		47. Aseptic necrosis	☐Yes ☐No

Cardiovascular & Respiratory - MUST BE SLE RELATED		Renal - MUST BE SLE RELATED		(√) if SLE Related
48. Pleuropericardial pain	0 1 2 3 4	68. Systolic Blood Pressure (Enter value)	_____mm-Hg	☐
49. Dyspnea	0 1 2 3 4	69. Diastolic Blood Pressure (Enter value)	_____mm-Hg	☐
50. Cardiac Failure	0 1 2 3 4	70. Accelerated Hypertension	☐Yes ☐No	☐
51. Friction Rub	0 1 2 3 4	71. Urine dipstick (Enter value) (- = 0) (+ = 1) (++ = 2) (+++ = 3)		☐
52. Effusion (pericardial or pleural)	0 1 2 3 4	72. Urinary protein (Record a or b) a. 24 hr urinary protein b. Urine protein-creatine ratio	a _____ g b _____mm/mmol	☐
53. Mild or intermittent chest pain	0 1 2 3 4	73. Proteinuria (Record a or b) a. Newly documented proteinuria of > 1g/24 hours b. Newly documented protein-creatine ratio of >100mg/mmol	a. ☐Yes ☐No b. ☐Yes ☐No	
54. Progressive CXR changes – lung fields *If Not Done,√ NO on EDC BILAG	☐Yes OR Circle: No / Not Done	74. Nephrotic Syndrome	☐Yes ☐No	☐
55. Progressive CXR changes – heart size *If Not Done,√ NO on EDC BILAG	☐Yes OR Circle No / Not Done	75. Creatinine (serum) (Enter value)	_____mg/dl	☐
56. ECG evidence of pericarditis or Myocarditis *If Not Done,√ NO on EDC BILAG	☐Yes OR Circle No / Not Done	76. Creatinine clearance/GFR (Enter value)	_____ml/min	☐
57. Cardiac dysrhythmias including tachycardia >100 in the absence of fever *If Not Done,√ NO on EDC BILAG	☐Yes OR Circle No / Not Done	77. Active urinary sediment	☐Yes ☐No	☐
58. Pulmonary function fall by 20% *If Not Done,√ NO on EDC BILAG	☐Yes OR Circle No / Not Done	78. Histological evidence of active Nephritis - within 3 months	☐Yes ☐No	
59. Cytohistological evidence of inflammatory lung disease *If Not Done,√ NO on EDC BILAG	☐Yes OR Circle No / Not Done	86. Evidence of circulating anticoagulant	☐Yes ☐No	
Vascular - MUST BE SLE RELATED		**Hematology - MUST BE SLE RELATED**		
60. Major cutaneous vasculitis incl. ulcers	0 1 2 3 4	79. Hemoglobin (g/dl) (Enter value)	_____g/dl	☐
61. Major abdominal crisis due to vasculitis	0 1 2 3 4	80. Total white cell count (x 10^9/L) (Enter value)	_____x 10^9/L	☐
62. Recurrent thromboembolism excluding strokes	0 1 2 3 4	81. Neutrophils (x 10^9/L) (Enter value)	_____x 10^9/L	☐
63. Raynaud's	0 1 2 3 4	82. Lymphocytes (x 10^9/L) (Enter value)	_____x 10^9/L	☐
64. Livido reticularis	0 1 2 3 4	83. Platelets (x 10^9/L) (Enter value)	_____x 10^9/L	☐
65. Superficial phlebitis	0 1 2 3 4	84. Evidence of active hemolysis	☐Yes ☐No	☐
66. Minor cutaneous vasculitis (nailfold vascultitis, digital vasculitis)	0 1 2 3 4	85. Coombs test positive	☐Yes ☐No	
67. Thromboembolism (excl. stroke) (first episode)	☐Yes ☐No	86. Evidence of circulating anticoagulant	☐Yes ☐No	

Appendix L: Systemic Lupus International Collaborating Clinics (SLICC)

System Lupus International Collaborating Clinics/American College of Rheumatology Damage Index for Systemic Lupus Erythematosus*

Item	Score
Ocular (either eye, by clinical assessment)	
Any cataract ever	1
Retinal change or optic atrophy	1
Neuropsychiatric	
Cognitive impairment (e.g. memory deficit, difficulty with calculation, poor concentration, difficulty in spoken or written language, impaired performance levels) or major psychosis	1
Seizures requiring therapy for 6 months	1
Cerebrovascular accident ever (score 2 > 1)	1 (2)
Cranial or peripheral neuropathy (excluding optic)	1
Transverse myelitis	1
Renal	
Estimated or measured glomerular filtration rate<50%	1
Proteinuria ≥3.5 gm/24hours	1
Or	
End-stage renal disease (regardless of dialysis or transplantation)	3
Pulmonary	
Pulmonary hypertension (right ventricular prominence, or loud P2)	1
Pulmonary fibrosis (physical and radiograph)	1
Shrinking lung (radiograph)	1
Pleural fibrosis (radiograph)	1
Pulmonary infarction (radiograph)	1
Cardiovascular	
Angina or coronary artery bypass	1
Myocardial infarction ever (score 2 if > 1)	1(2)
Cardiomyopathy (ventricular dysfunction)	1
Valvular disease (diastolic murmur, or systolic murmur >3/6)	1
Pericarditis for 6 months, or pericardiectomy	1
Peripheral vascular	
Claudication for 6 months	1
Minor tissue loss (pulp space)	1
Significant tissue loss ever (e.g. loss of digit or limb)(score 2 if > 1 site)	1(2)
Venous thrombosis with swelling, ulceration, or venous stasis	1
Gastrointestinal	
Infarction or resection of bowel below duodenum spleen, liver, or gall bladder ever, for cause any (score 2 if > 1 site)	1(2)
Mesenteric insufficiency	1
Chronic peritonitis	1
Stricture or upper gastrointestinal tract surgery ever	1
Musculoskeletal	
Muscle atrophy or weakness	1
Deforming or erosive arthritis (including reducible deformities, excluding avascular necrosis)	1
Osteoporosis with fracture or vertebral collapse (excluding avascular necrosis)	1
Avascular necrosis (score 2 if > 1)	1(2)
Osteomyelitis	1
Skin	
Scarring chronic alopecia	1
Extensive scarring or panniculum other than scalp and pulp space	1
Skin ulceration (excluding thrombosis) for > 6 months	1
Premature gonadal failure	1
Diabetes (regardless of treatment)	1
Malignancy (exclude dysplasia) (score 2 if > 1 site)	1(2)

*Damage (nonreversible change, not related to active inflammation) occurring since onset of lupus, ascertained by clinical assessment and present for at least **6 months** unless otherwise stated. Repeat episodes must occur 6 months apart to score 2. The same lesion cannot be scored twice.

Appendix M: Fatigue Severity Scale (FSS)

Neuroimaging Sciences
University of Edinburgh
based at DCN
Western General Hospital
Crewe Road
Edinburgh EH4 2XU

Principal Investigator: Stewart Wiseman swiseman@staffmail.ed.ac.uk Tel: 0131 537 2660 or 0131 537 1985

Fatigue Severity Scale

Patient Code: [ ] Patient Initials: [ ]

Please circle the number between 1 and 7 which you feel best fits the following statements. This refers to your usual way of life within the last week.

1 indicates "strongly disagree" and 7 indicates "strongly agree."

	Strongly Disagree						Strongly Agree
1. My motivation is lower when I am fatigued.	1	2	3	4	5	6	7
2. Exercise brings on my fatigue.	1	2	3	4	5	6	7
3. I am easily fatigued.	1	2	3	4	5	6	7
4. Fatigue interferes with my physical functioning	1	2	3	4	5	6	7
5. Fatigue causes frequent problems for me.	1	2	3	4	5	6	7
6. My fatigue prevents sustained physical functioning.	1	2	3	4	5	6	7
7. Fatigue interferes with carrying out certain duties and responsibilities.	1	2	3	4	5	6	7
8. Fatigue is among my most disabling symptoms.	1	2	3	4	5	6	7
9. Fatigue interferes with my work, family, or social life.	1	2	3	4	5	6	7

Fatigue Severity Scale

Patient Code: [ ] Patient Initials: [ ]

Add up later

Scoring: Add up the 9 scores and divide by 9

FSS Score:

[ ]

Scores range from 1 to 7
Higher scores indicate more fatigue
For reference, normal healthily adults average 2.3 (SD ±0.7)

Appendix N: Hospital Anxiety and Depression Scale (HADS)

HADS

Patient Code: Patient Initials:

Please chose the column which best matches, then circle the number in that column →	Yes, definitely	Yes, sometimes	No, not much	No, not at all
1. I wake early and then sleep badly for the rest of the night.	3	2	1	0
2. I get very frightened or have panic feelings for apparently no reason at all.	3	2	1	0
3. I feel miserable and sad.	3	2	1	0
4. I feel anxious when I go out of the house on my own.	3	2	1	0
5. I have lost interest in things.	3	2	1	0
6. I get palpitations, or sensations of 'butterflies' in my stomach or chest.	3	2	1	0
7. I have a good appetite.	0	1	2	3
8. I feel scared or frightened.	3	2	1	0
9. I feel life is not worth living.	3	2	1	0
10. I still enjoy the things I used to.	0	1	2	3
11. I am restless and can't keep still.	3	2	1	0
12. I am more irritable than usual.	3	2	1	0
13. I feel as if I have slowed down.	3	2	1	0
14. Worrying thoughts constantly go through my mind.	3	2	1	0

HADS

Patient Code: [ ] Patient Initials: [ ]

Add up later

Anxiety	(add up highlighted questions 2,4,6,8,11,12,14)
Depression	(add up 1,3,5,7,9,10,13)
Scoring	3, 2, 1, 0 (For items 7 & 10 the scoring is reversed)

Anxiety Score:

[ ]

Depression Score:

[ ]

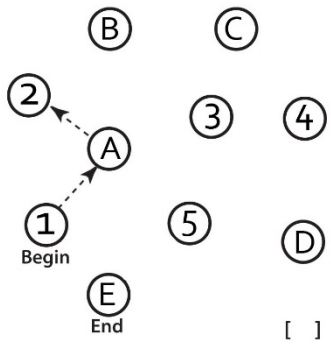
GRADING: 0 - 7 = Non-case 8 – 10 = Borderline case 11+ = Case

Appendix O: Montreal Cognitive Assessment (MoCA)

MONTREAL COGNITIVE ASSESSMENT (MOCA)
Version 7.3 Alternative Version

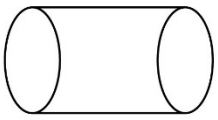
NAME :
Education : Date of birth :
Sex : DATE :

VISUOSPATIAL / EXECUTIVE — POINTS



[ ] [ ]

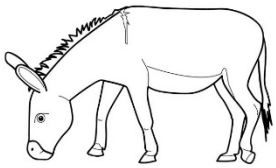
Copy cylinder

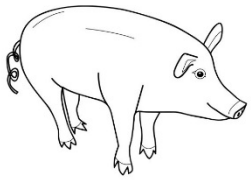


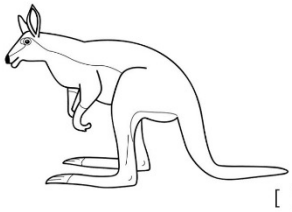
Draw CLOCK (Ten past nine)
(3 points)

[ ] Contour [ ] Numbers [ ] Hands __/5

NAMING __/3


[ ]


[ ]


[ ]

MEMORY Read list of words, subject must repeat them. Do 2 trials, even if 1st trial is successful. Do a recall after 5 minutes. — No points

	TRAIN	EGG	HAT	CHAIR	BLUE
1st trial					
2nd trial					

ATTENTION Read list of digits (1 digit/ sec.). Subject has to repeat them in the forward order [ ] 5 4 1 8 7
Subject has to repeat them in the backward order [ ] 1 7 4 __/2

Read list of letters. The subject must tap with his hand at each letter A. No points if ≥ 2 errors
[ ] F B A C M N A A J K L B A F A K D E A A A J A M O F A A B __/1

Serial 7 subtraction starting at 80 [ ] 73 [ ] 66 [ ] 59 [ ] 52 [ ] 45
4 or 5 correct subtractions: **3 pts**, 2 or 3 correct: **2 pts**, 1 correct: **1 pt**, 0 correct: **0 pt** __/3

LANGUAGE Repeat : She heard his lawyer was the one to sue after the accident. [ ]
The little girls who were given too much candy got stomach aches. [ ] __/2

Fluency / Name maximum number of words in one minute that begin with the letter B [ ] _____ (N ≥ 11 words) __/1

ABSTRACTION Similarity between e.g. banana - orange = fruit [ ] eye – ear [ ] trumpet – piano __/2

DELAYED RECALL __/5

	Has to recall words **WITH NO CUE**	TRAIN [ ]	EGG [ ]	HAT [ ]	CHAIR [ ]	BLUE [ ]	Points for UNCUED recall only
Optional	Category cue						
	Multiple choice cue						

ORIENTATION [ ] Date [ ] Month [ ] Year [ ] Day [ ] Place [ ] City __/6

Adapted by : Z. Nasreddine MD, N. Phillips PhD, H. Chertkow MD
 www.mocatest.org
Normal ≥ 26 / 30

TOTAL __/30
Add 1 point if ≤ 12 yr edu

Administered by: _____

Appendix P: Addenbrooke's Cognitive Examination – Revised (ACER)

ADDENBROOKE'S COGNITIVE EXAMINATION - ACE-R

Final Revised Version A (2005)

Name :
Date of birth :
Hospital no. :

Addressograph

Date of testing: ..... /..... /.....
Tester's name: .....
Age at leaving full-time education: .....
Occupation: .....
Handedness: .....

ORIENTATION — ATTENTION & ORIENTATION

- Ask: What is the | Day ..... | Date ..... | Month ..... | Year ..... | Season .....
- Ask: Which | Building ..... | Floor ..... | Town ..... | County ..... | Country .....

[Score 0-5] ☐☐

[Score 0-5] ☐☐

REGISTRATION

- Tell: 'I'm going to give you three words and i'd like you to repeat after me: lemon, key and ball'. After subject repeats, say 'Try to remember them because i'm going to ask you later'. Score only the first trial (repeat 3 times if necessary).

Register number of trials .....

[Score 0-3] ☐☐

ATTENTION & CONCENTRATION

- Ask the subject: ' could you take 7 away from a 100? After the subject responds, ask him or her to take away another 7 to a total of 5 subtractions. If subject make a mistake, carry on and check the subsequent answer (i.e. 93, 84, 77, 70, 63 -score 4)

Stop after five subtractions (93, 86, 79, 72, 65). ....

- Ask: 'could you please spell **WORLD** for me? Then ask him/her to spell it backwards:

....

[Score 0-5] ☐☐
(for the best performed task)

MEMORY - Recall — MEMORY

- Ask: 'Which 3 words did I ask you to repeat and remember?'

....

[Score 0-3] ☐☐

MEMORY - Anterograde Memory

- Tell: ' I'm going to give you a name and address and I'd like you to repeat after me. We'll be doing that 3 times, so you have a chance to learn it. I'll be asking you later'

Score only the third trial

[Score 0-7] ☐

	1st Trial	2nd Trial	3rd Trial
Harry Barnes	....	....	....
73 Orchard Close	....	....	....
Kingsbridge	.....	.....	.....
Devon	.....	.....	.....

MEMORY - Retrograde Memory

- Name of current Prime Minister .....
- Name of the woman who was Prime Minister .....
- Name of the USA president .....
- Name of the USA president who was assassinated in the 1960's .....

[Score 0 -4] ☐

ADDENBROOKE'S COGNITIVE EXAMINATION - ACE-R *Final Revised Version (2005)*

VERBAL FLUENCY - Letter 'P' and animals

➢ **Letters**

Say: 'I'm going to give you a letter of the alphabet and I'd like you to generate as many words as you can beginning with that letter, but not names of people or places. Are you ready? You've got a minute and the letter is P'

[Score 0 - 7] ☐

				>17	7
				14-17	6
				11-13	5
				8-10	4
				6-7	3
				4-5	2
				2-3	1
				<2	0
				total	correct

➢ **Animals**

Say: 'Now can you name as many animals as possible, beginning with any letter?

[Score 0 - 7] ☐

				>21	7
				17-21	6
				14-16	5
				11-13	4
				9-10	3
				7-8	2
				5-6	1
				<5	0
				total	correct

FLUENCY

LANGUAGE - Comprehension

➢ Show written instruction:

[Score 0-1] ☐ ☐

Close your eyes

➢ 3 stage command:
'Take the paper in your right hand. Fold the paper in half. Put the paper on the floor'

[Score 0-3] ☐ ☐

LANGUAGE - Writing

➢ Ask the subject to make up a sentence and write it in the space below:
Score 1 if sentence contains a subject and a verb (see guide for examples)

[Score 0-1] ☐ ☐

LANGUAGE

ADDENBROOKE'S COGNITIVE EXAMINATION - ACE-R *Final Revised Version (2005)*

LANGUAGE - Repetition

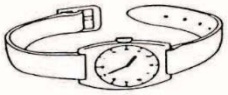
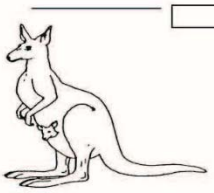
➢ Ask the subject to repeat:**' hippopotamus'; 'eccentricity; 'unintelligible'; 'statistician'**
Score 2 if all correct; 1 if 3 correct; 0 if 2 or less. [Score 0-2] ☐

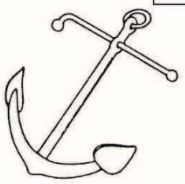
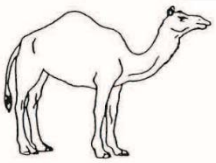
➢ Ask the subject to repeat: **'Above, beyond and below'** [Score 0-1] ☐

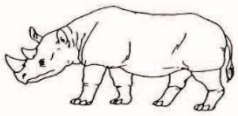
➢ Ask the subject to repeat: **'No ifs, ands or buts'** [Score 0-1] ☐

LANGUAGE - Naming

➢ Ask the subject to name the following pictures: [Score 0-2] pencil + watch ☐




[Score 0-10] ☐

LANGUAGE - Comprehension

➢ Using the pictures above, ask the subject to: [Score 0-4] ☐

- Point to the one which is associated with the monarchy _____
- Point to the one which is a marsupial _____
- Point to the one which is found in the Antarctic _____
- Point to the one which has a nautical connection _____

LANGUAGE

ADDENBROOKE'S COGNITIVE EXAMINATION - ACE-R *Final Revised Version (2005)*

LANGUAGE - Reading

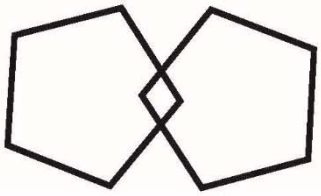
➢ Ask the subject to read the following words: [Score 1 only if all correct] [Score 0-1]

sew
pint
soot
dough
height

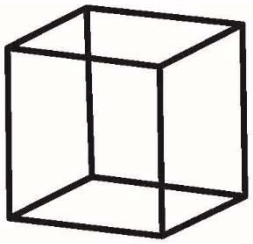
LANGUAGE

VISUOSPATIAL ABILITIES

➢ Overlapping pentagons: Ask the subject to copy this diagram: [Score 0-1]



➢ Wire cube : Ask the subject to copy this drawing (for scoring, see instructions guide) [Score 0-2]



➢ Clock: Ask the subject to draw a clock face with numbers and the hands at ten past five. (for scoring see instruction guide: circle = 1, numbers = 2, hands = 2 if all correct) [Score 0-5]

VISUOSPATIAL

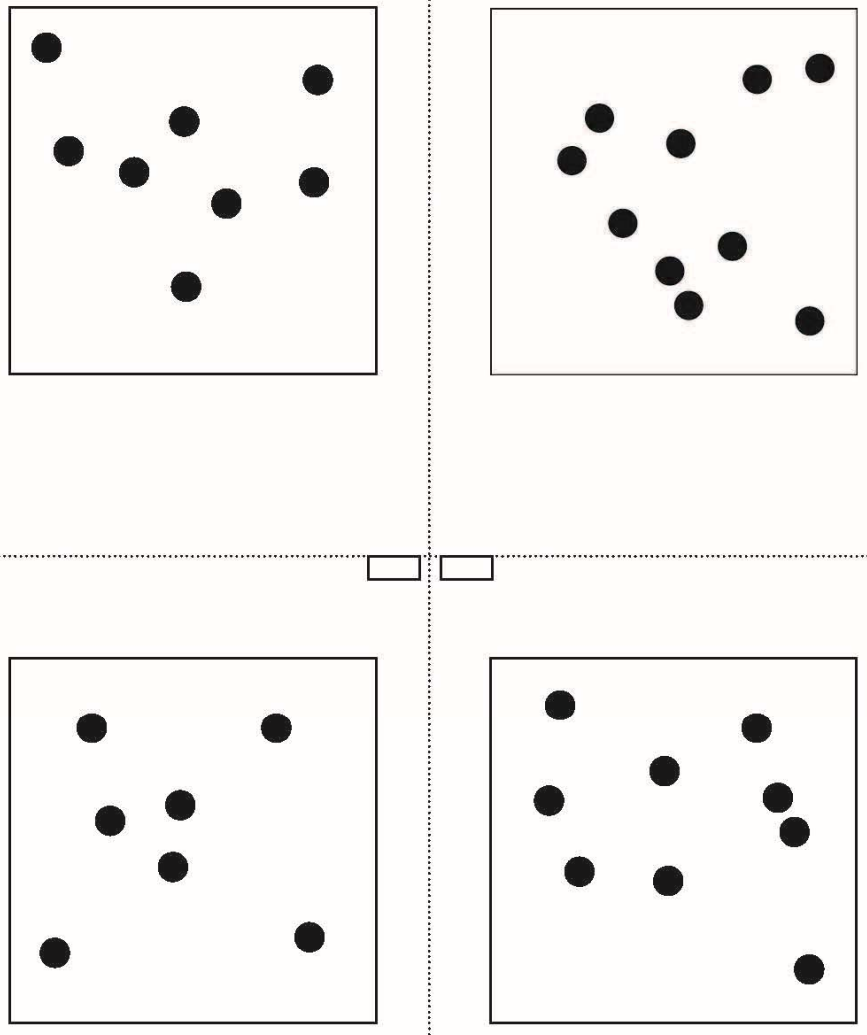
ADDENBROOKE'S COGNITIVE EXAMINATION - ACE-R

Final Revised Version (2005)

PERCEPTUAL ABILITIES

➢ Ask the subject to count the dots without pointing them

[Score 0-4]



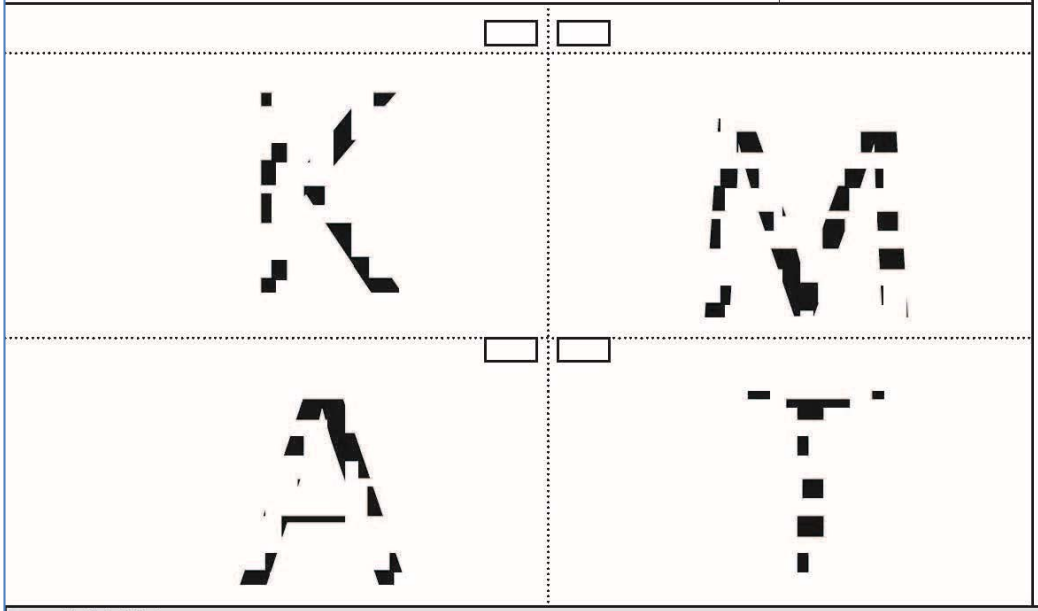
VISUOSPATIAL

ADDENBROOKE'S COGNITIVE EXAMINATION - ACE-R

Final Revised Version A (2005)

PERCEPTUAL ABILITIES

➤ Ask the subject to identify the letters [Score 0-4]



RECALL

➤ Ask "Now tell me what you remember of that name and address we were repeating at the beginning'"

		[Score 0-7]
Harry Barnes	....	
73 Orchard Close	....	
Kingsbridge	.....	
Devon	.....	

RECOGNITION

➤ This test should be done if subject failed to recall one or more items. If all items were recalled, skip the test and score 5. If only part is recalled start by ticking items recalled in the shadowed column on the right hand side. Then test not recalled items by telling "ok, I'll give you some hints: was the name X, Y or Z?" and so on. Each recognised item scores one point which is added to the point gained by recalling. [Score 0-5]

Jerry Barnes		Harry Barnes		Harry Bradford		recalled	
37		73		76		recalled	
Orchard Place		Oak Close		Orchard Close		recalled	
Oakhampton		Kingsbridge		Dartington		recalled	
Devon		Dorset		Somerset		recalled	

General Scores

MMSE	/30
ACE-R	/100

Subscores

Attention and Orientation	/18
Memory	/26
Fluency	/14
Language	/26
Visuospatial	/16

VISUOSPATIAL MEMORY SCORE

Normative values based on 63 controls aged 52-75 and 142 dementia patients aged 46-86

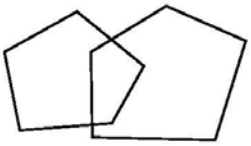
Cut-off <88 gives 94% senstivity and 89% specificity for dementia
Cut-off <82 gives 84% sensitivity and 100% specificity for dementia

Appendix Q: Mini Mental State Examination (MMSE)

MINI MENTAL STATE EXAMINATION (MMSE)

Patient's name:

Hospital number:

ONE POINT FOR EACH ANSWER	DATE			
ORIENTATION				
Year Month Day Date Time	____/5	____/5	____/5	____/5
Country Town District Hospital Ward	____/5	____/5	____/5	____/5
REGISTRATION Examiner names 3 objects (eg apple, table, penny) Patient asked to repeat (1 point for each correct). THEN patient to learn the 3 names repeating until correct.	____/3	____/3	____/3	____/3
ATTENTION AND CALCULATION Subtract 7 from 100, then repeat from result. Continue 5 times: 100 93 86 79 65 Alternative: spell "WORLD" backwards - dlrow.	____/5	____/5	____/5	____/5
RECALL Ask for names of 3 objects learned earlier.	____/3	____/3	____/3	____/3
LANGUAGE Name a pencil and watch.	____/2	____/2	____/2	____/2
Repeat "No ifs, ands, or buts".	____/1	____/1	____/1	____/1
Give a 3 stage command. Score 1 for each stage. Eg. "Place index finger of right hand on your nose and then on your left ear".	____/3	____/3	____/3	____/3
Ask patient to read and obey a written command on a piece of paper stating "Close your eyes".	____/1	____/1	____/1	____/1
Ask the patient to write a sentence. Score if it is sensible and has a subject and a verb.	____/1	____/1	____/1	____/1
COPYING Ask the patient to copy a pair of intersecting pentagons: 	____/1	____/1	____/1	____/1
TOTAL	____/30	____/30	____/30	____/30

Appendix R: National Adult Reading Test (NART)

NART = National Adult Reading Test (a proxy for childhood IQ – came about because it has been noticed that people with dementia are still able to read surprisingly well)

1. Give participant the laminated card to read from.
2. Radiographer has a paper copy to score on (mark **incorrect** pronunciations and add up later)
3. Explain to the participant that this is a hard test, and not to worry. Explain that the words get harder as you go down the list. Tell the participant that the words cannot be '*worked out*' – you either know them or you don't and that is the point of the test.
4. Radiographers should be thoroughly familiar with correct pronunciation before testing someone
5. Ask the participant to go slowly and if necessary ask them to wait (eg, if you have trouble keeping up with the scoring). There is no time limit.
6. Encourage participant to attempt every word. Make a note of how many attempted if participant wants to abandon test part way through.
7. All responses should be re-inforced with "that's fine, good" etc, especially if they seem anxious.
8. Participants are allowed to change their mind, but must give a final opinion on pronunciation.

CHORD	SUPERFLUOUS
ACHE	SIMILE
DEPOT	BANAL
AISLE	QUADRUPED
BOUQUET	CELLIST
PSALM	FACADE
CAPON	ZEALOT
DENY	DRACHM
NAUSEA	AEON
DEBT	PLACEBO
COURTEOUS	ABSTEMIOUS
RAREFY	DETENTE
EQUIVOCAL	IDYLL
NAIVE	PUERPERAL
CATACOMB	AVER
GAOLED	GAUCHE
THYME	TOPIARY
HEIR	LEVIATHAN
RADIX	BEATIFY
ASSIGNATE	PRELATE
HIATUS	SIDEREAL
SUBTLE	DEMESNE
PROCREATE	SYNCOPE
GIST	LABILE
GOUGE	CAMPANILE

Patient Code:

Avg number of errors is 22.4 ± 10.1.

NART **error** score out of 50: